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* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *
FILE 'HOME' ENTERED AT 10:32:32 ON 15 MAR 2004
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e watson james d/au
E1            3     WATSON JAMES CHESLEY/AU
E2            2     WATSON JAMES CLYDE/AU
E3          256 --> WATSON JAMES D/AU
E4            3     WATSON JAMES DEWEY/AU
E5           12     WATSON JAMES DOUGLAS/AU
E6           60     WATSON JAMES E/AU
E7           25     WATSON JAMES E JR/AU
E8            1     WATSON JAMES EDWIN/AU
E9            1     WATSON JAMES ELWYN JR/AU
E10          12     WATSON JAMES F/AU
E11           2     WATSON JAMES FREDERICK/AU
E12           1     WATSON JAMES FREDRICK II/AU
=> s e3-e5 and vacca?
L1           21 ("WATSON JAMES D"/AU OR "WATSON JAMES DEWEY"/AU OR "WATSON JAMES
                DOUGLAS"/AU) AND VACCA?
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           13 DUP REM L1 (8 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 13 ANSWERS - CONTINUE? Y/(N):y
L2   ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2004 ACS on STN DUPLICATE 1
AN   2003:23361  CAPLUS
DN   138:88641
TI   Mycobacterium   ***vaccae***   antigens for treating immunologically
     mediated skin disorders
IN     ***Watson, James D.***  ; Tan, Paul L. J.; Prestidge, Ross
PA   N. Z.
SO   U.S. Pat. Appl. Publ., 122 pp., Cont.-in-part of U.S. 6,328,978.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 8
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   US 2003007976      A1   20030109       US 2001-880505   20010613
     US 5968524         A    19991019       US 1997-997080   19971223
     US 6328978         B1   20011211       US 1999-324542   19990602
PRAI US 1997-997080     A2   19971223
     US 1999-324542     A2   19990602
AB   Methods for the treatment of skin disorders, including psoriasis, atopic
     dermatitis, allergic contact dermatitis, alopecia areata, skin cancers,
     and related disorders, such as psoriatic arthritis are provided, such
     methods comprising administering a compn. having antigenic and/or adjuvant
     properties.  Compns. which may be usefully employed in the inventive
     methods include inactivated M.   ***vaccae***   cells, delipidated and
     deglycolipidated M.   ***vaccae***   cells, M.   ***vaccae***   culture
     filtrate and compds. present in or derived therefrom, together with
     combinations of such compns.

L2   ANSWER 2 OF 13  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2003:133100  CAPLUS
DN   138:186385
TI   Inactivated Mycobacterium   ***vaccae***   and antigens for modulating
     immune responses and treating autoimmune disease, allergy and graft
     rejection
IN     ***Watson, James D.***  ; Tan, Paul L. J.; Abernethy, Nevin
PA   Genesis Research and Development Corporation Limited, N. Z.
SO   PCT Int. Appl., 136 pp.
     CODEN: PIXXD2
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DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003013595	A1	20030220	WO 2002-NZ135	20020726

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	US 2003147861	A1	20030807	US 2002-205979	20020725

PRAI US 2001-308446P P 20010726

AB Methods and compns. for the modification of immune response by modulating the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. The components include inactivated Mycobacterium ***vaccae*** ; delipidated or deglycolipidated Mycobacterium ***vaccae*** ; acid or alk. hydrolysis-treated delipidated or deglycolipidated Mycobacterium ***vaccae*** ; or Mycobacterium ***vaccae*** antigens GV-1/70, GV-1/83, GV-3, GV-4P, GV-5, GV-5P, GV--7, GV-9, GV-13, GV-14, GV-22B, GV-23, GV-24B, GV-27, GV-27A, GV-27B, GV-29, GV-33, GV-35, GV-38AP, GV-38BP, GV-40P, GV-41B, GV-42, GV-44 and GV-45. The unwanted immune response or disorders are autoimmune diseases, allergic disorders and graft rejection.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 13 USPATFULL on STN
AN 2003:213224 USPATFULL
TI Compounds and methods for the modulation of immune responses
IN ***Watson, James D.*** , Auckland, NEW ZEALAND
Tan, Paul L.J., Bondi Junction, AUSTRALIA
Abernethy, Nevin, Auckland, NEW ZEALAND
PA Genesis Research and Development Corporation Limited, Auckland, NEW ZEALAND (non-U.S. corporation)
PI US 2003147861 A1 20030807
AI US 2002-205979 A1 20020725 (10)
PRAI US 2001-308446P 20010726 (60)
DT Utility
FS APPLICATION
LREP SPECKMAN LAW GROUP, 1501 WESTERN AVE, SUITE 100, SEATTLE, WA, 98101
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 29 Drawing Page(s)
LN.CNT 3933
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and compositions for the modification of immune response by modulating of the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. Such compositions comprise components derived from Mycobacteria, such as Mycobacterium ***vaccae*** .

L2 ANSWER 4 OF 13 USPATFULL on STN
AN 2003:30886 USPATFULL
TI Compositions isolated from skin cells and methods for their use
IN ***Watson, James D.*** , Auckland, NEW ZEALAND

Strachan, Lorna, Auckland, NEW ZEALAND
Sleeman, Matthew, Weston Colville, UNITED KINGDOM
Onrust, Rene, Mercer Is, WA, UNITED STATES
Murison, James G., Auckland, NEW ZEALAND
Kumble, Krishanand D., Los Altos, CA, UNITED STATES
PA Genesis Research and Development Corporation Limited, Auckland, NEW ZEALAND (non-U.S. corporation)
PI US 2003022835 A1 20030130
AI US 2002-152661 A1 20020520 (10)
RLI Continuation-in-part of Ser. No. US 2001-866050, filed on 24 May 2001, PENDING Continuation-in-part of Ser. No. US 1999-312283, filed on 14 May 1999, PENDING Continuation-in-part of Ser. No. US 1998-188930, filed on 9 Nov 1998, GRANTED, Pat. No. US 6150502 Continuation-in-part of Ser. No. US 1998-69726, filed on 29 Apr 1998, ABANDONED
PRAI WO 1999-NZ51 19990429
US 2000-206650P 20000524 (60)
US 2000-221232P 20000725 (60)
DT Utility
FS APPLICATION
LREP SPECKMAN LAW GROUP, 1501 WESTERN AVE, SUITE 100, SEATTLE, WA, 98101
CLMN Number of Claims: 29
ECL Exemplary Claim: 1
DRWN 14 Drawing Page(s)
LN.CNT 3053
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated polynucleotides encoding polypeptides expressed in mammalian skin cells are provided, together with expression vectors and host cells comprising such isolated polynucleotides. Methods for the use of such polynucleotides and polypeptides are also provided.

L2 ANSWER 5 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 2
AN 2003:346626 BIOSIS
DN PREV200300346626
TI A genome-based functional screening approach to vaccine development that combines in vitro assays and DNA immunization.
AU Delcayre, Alain [Reprint Author]; Peake, John S.; White, Damian J.; Yuan, Shining; McDonald, Megan K.; Liang, Andrew; Tan, Paul L.; ***Watson,*** *** James D.***
CS Anosys Inc., 1014 Hamilton Court, Menlo Park, CA, 94025, USA
adelcayre@anosys.com
SO Vaccine, (4 July 2003) Vol. 21, No. 23, pp. 3259-3264. print.
ISSN: 0264-410X (ISSN print).
DT Article
LA English
ED Entered STN: 30 Jul 2003
Last Updated on STN: 30 Jul 2003
AB A two-step screening strategy was developed to identify strong immunogenic polypeptides with putative vaccine andlor adjuvant activity. In the first step, a mycobacterial genomic DNA library was screened in vitro to identify plasmids encoding polypeptides that stimulate splenocytes from mycobacteria-immunized mice and T cells from PPD-positive healthy donors to produce interferon-gamma. In the second step, plasmids were selected for their ability to induce protective immunity in a mouse model of tuberculosis following DNA immunization. The potential of this approach is illustrated by the identification of a panel of immunogenic polypeptides that may be used to engineer a new generation of vaccines.

L2 ANSWER 6 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 3
AN 2002:401976 BIOSIS
DN PREV200200401976
TI Compounds and methods for treatment and diagnosis of mycobacterial

infections.
AU Tan, Paul L. [Inventor, Reprint author]; Visser, Elizabeth [Inventor]; Prestidge, Ross [Inventor]; ***Watson, James D.*** [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corporation Limited, New Zealand
PI US 6406704 June 18, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 18, 2002) Vol. 1259, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 24 Jul 2002
Last Updated on STN: 24 Jul 2002
AB The present invention provides polypeptides comprising an immunogenic portion of a M. ***vaccae*** protein and DNA molecules encoding such polypeptides, together with methods for their use in the diagnosis and treatment of mycobacterial infection. Methods for enhancing the immune response to an antigen including administration of M. ***vaccae*** culture filtrate, delipidated M. ***vaccae*** cells or delipidated and deglycolipidated M. ***vaccae*** cells are also provided.

L2 ANSWER 7 OF 13 USPATFULL on STN
AN 2002:343542 USPATFULL
TI Methods and compounds for the treatment of immunologically - mediated diseases of the respiratory system using mycobacterium ***vaccae***
IN ***Watson, James D.*** , Auckland, NEW ZEALAND
Tan, Paul L.J., Auckland, NEW ZEALAND
PI US 2002197265 A1 20021226
AI US 2002-51643 A1 20020118 (10)
RLI Continuation of Ser. No. US 1998-156181, filed on 17 Sep 1998, PENDING Continuation-in-part of Ser. No. US 1997-996624, filed on 23 Dec 1997, ABANDONED
DT Utility
FS APPLICATION
LREP Janet Sleath, SPECKMAN LAW GROUP, Suite 100, 1501 Western Avenue, Seattle, WA, 98101
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 10 Drawing Page(s)
LN.CNT 6136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment by immunotherapy of lung immune disorders, including infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such components.

L2 ANSWER 8 OF 13 USPATFULL on STN
AN 2002:39664 USPATFULL
TI Methods and compounds for the treatment of immunologically-mediated diseases using mycobacterium ***vaccae***
IN ***Watson, James D.*** , Auckland, NEW ZEALAND
Tan, Paul L. J., Auckland, NEW ZEALAND
Prestidge, Ross, Auckland, NEW ZEALAND
PA Genesis Research & Development Corporation Limited, NEW ZEALAND (non-U.S. corporation)
PI US 6350457 B1 20020226

AI US 1999-449013 19991124 (9)
PRAI US 1999-137112P 19990602 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P
LREP Sleath, Janet, Speckman, Ann W.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1305
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a composition comprising derivatives of delipidated and deglycolipidated M ***vaccae*** cells.

L2 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 2002:113776 BIOSIS
DN PREV200200113776
TI Methods for the treatment of immunologically-mediated skin disorders.
AU ***Watson, James D.*** [Inventor, Reprint author]; Tan, Paul L. J. [Inventor]; Prestidge, Ross [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corp. Ltd., Parnell, New Zealand
PI US 6328978 December 11, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 11, 2001) Vol. 1253, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 30 Jan 2002
Last Updated on STN: 26 Feb 2002
AB Methods for the treatment of skin disorders, including psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata and skin cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such compositions.

L2 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:880993 CAPLUS
DN 134:41096
TI Methods and compounds for the treatment of immunologically-mediated diseases using Mycobacterium ***vaccae***
IN ***Watson, James D.*** ; Tan, Paul L. J.; Prestidge, Ross L.
PA Genesis Research & Development Corporation Limited, N. Z.
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000074715	A1	20001214	WO 2000-NZ85	20000601

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,

ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6350457	B1	20020226	US 1999-449013	19991124	
EP 1181051	A1	20020227	EP 2000-937399	20000601	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, MC, PT, IE, SI, LT, LV, FI, RO					
BR 2000011239	A	20020402	BR 2000-11239	20000601	
JP 2003501400	T2	20030114	JP 2001-501249	20000601	
PRAI US 1999-137112P	P	19990602			
US 1999-449013	A	19991124			
WO 2000-NZ85	W	20000601			

AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as (M. tuberculosis) or (M. avium), sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a compn. comprising at least one deriv. of delipidated and deglycolipidated (M. ***vaccae***) cells.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 11 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:140561 BIOSIS
DN PREV200000140561
TI Improvement in psoriasis after intradermal administration of heat-killed Mycobacterium ***vaccae*** .
AU Balagon, Maria V.; Walsh, Douglas S. [Reprint author]; Tan, Paul L.; Cellona, Roland V.; Abalos, Rodolfo M.; Tan, Esterlina V.; Fajardo, Tranquilino T., Jr.; ***Watson, James D.*** ; Walsh, Gerald P.
CS Armed Forces Research Institute of Medical Sciences (AFRIMS), APO, AP, 96546-5000, USA
SO International Journal of Dermatology, (Jan., 2000) Vol. 39, No. 1, pp. 51-58. print.
CODEN: IJDEBB. ISSN: 0011-9059.
DT Article
LA English
ED Entered STN: 19 Apr 2000
Last Updated on STN: 4 Jan 2002
AB Background: New treatments for psoriasis are being developed, but many are associated with limited efficacy, side-effects, or rapid recurrence after discontinuation. Thus, the aim of new agents is to induce longer term remissions with fewer side-effects. Preliminary studies have shown that Mycobacterium ***vaccae*** , a nonpathogenic organism prepared as a heat-killed suspension, may induce periods of remission in some psoriasis patients when administered intradermally. Methods: To further assess the efficacy and tolerability of M. ***vaccae*** in patients with moderate to severe psoriasis (psoriasis area and severity index (PASI) of 12-35), we conducted an open label study whereby 24 patients received two intradermal inoculations of M. ***vaccae*** in lesion-free deltoid skin, separated by a period of 3 weeks. Results: Twelve weeks after starting treatment, 14 of 24 patients (58%) showed marked improvement in the PASI score (greater than 50% reduction), two had moderate improvement (25-50% reduction), six were unchanged (< 25% reduction), and two had worsened (> 5% increase). By 24 weeks, 11 of 22 patients continued to show greater than 50% improvement. Five patients had complete clearance of skin lesions that lasted for at least 6 months. Conclusions: Intradermal administration of heat-killed M. ***vaccae*** suspension was well tolerated and induced clinically significant improvement in a majority of psoriasis patients in this cohort. Placebo-controlled testing

to further define the efficacy of this treatment is warranted.

L2 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 2000:283534 BIOSIS
DN PREV200000283534
TI Methods and compounds for the treatment of immunologically-mediated psoriasis.
AU ***Watson, James D.*** [Inventor, Reprint author]; Tan, Paul L. J. [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corp., Auckland, New Zealand
PI US 5968524 October 19, 1999
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 19, 1999) Vol. 1227, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002
AB Methods for the treatment of skin disorders, including psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata and skin cancers are provided, such methods comprising administering multiple doses of a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such compositions.

L2 ANSWER 13 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1997:515428 BIOSIS
DN PREV199799814631
TI Immunization with heat-killed Mycobacterium ***vaccae*** stimulates CD8+ cytotoxic T cells specific for macrophages infected with Mycobacterium tuberculosis.
AU Skinner, Margot A.; Yuan, Shining; Prestidge, Ross; Chuk, David; ***Watson, James D.*** ; Tan, Paul L. J. [Reprint author]
CS Genesis Res. Dev. Corp. Ltd., PO Box 50, Auckland, New Zealand
SO Infection and Immunity, (1997) Vol. 65, No. 11, pp. 4525-4530.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 10 Dec 1997
Last Updated on STN: 10 Dec 1997
AB Immune responses to Mycobacterium tuberculosis are analyzed in mice which have been immunized with Mycobacterium ***vaccae*** to examine novel ways of altering protective immunity against M. tuberculosis. The spleen cells of mice immunized with M. ***vaccae*** proliferate and secrete gamma interferon (IFN-gamma) in response to challenge with live M. tuberculosis in vitro. Immunization with M. ***vaccae*** results in the generation of CD8+ T cells which kill syngeneic macrophages infected with M. tuberculosis. These effector cytotoxic T cells (CTL) are detectable in the spleen at 2 weeks after immunization with M. ***vaccae*** but cannot be found in splenocytes 3 to 6 weeks postimmunization. However, M. tuberculosis-specific CTL are revealed following restimulation in vitro with heat-killed M. ***vaccae*** or M. tuberculosis, consistent with the activation of memory cells. These CD8+ T cells secrete IFN-gamma and enhance the production of interleukin 12 when cocultured with M. tuberculosis-infected macrophages. It is suggested that CD8+ T cells with a cytokine secretion profile of the Tc1 class may themselves maintain the dominance of a Th1-type cytokine

response following immunization with M. ***vaccae*** . Heat-killed M. ***vaccae*** deserves attention as an alternative to attenuated live mycobacterial vaccines.

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=> e tan paul l j/au
=> s e2-e4 and vacca?
L3          21 ("TAN PAUL L"/AU OR "TAN PAUL L J"/AU OR "TAN PAUL LJ"/AU) AND
               VACCA?
=> dup rem l3
PROCESSING COMPLETED FOR L3
L4           15 DUP REM L3 (6 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 15 ANSWERS - CONTINUE? Y/(N):y
L4   ANSWER 1 OF 15  CAPLUS  COPYRIGHT 2004 ACS on STN DUPLICATE 1
AN   2003:23361  CAPLUS
DN   138:88641
TI   Mycobacterium   ***vaccae***   antigens for treating immunologically
     mediated skin disorders
IN   Watson, James D.;   ***Tan, Paul L. J.***  ; Prestidge, Ross
PA   N. Z.
SO   U.S. Pat. Appl. Publ., 122 pp., Cont.-in-part of U.S. 6,328,978.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 8
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   US 2003007976      A1   20030109       US 2001-880505   20010613
     US 5968524         A    19991019       US 1997-997080   19971223
     US 6328978         B1   20011211       US 1999-324542   19990602
PRAI US 1997-997080     A2   19971223
     US 1999-324542     A2   19990602
AB   Methods for the treatment of skin disorders, including psoriasis, atopic
     dermatitis, allergic contact dermatitis, alopecia areata, skin cancers,
     and related disorders, such as psoriatic arthritis are provided, such
     methods comprising administering a compn. having antigenic and/or adjuvant
     properties.  Compns. which may be usefully employed in the inventive
     methods include inactivated M.   ***vaccae***   cells, delipidated and
     deglycolipidated M.   ***vaccae***   cells, M.   ***vaccae***   culture
     filtrate and compds. present in or derived therefrom, together with
     combinations of such compns.

L4   ANSWER 2 OF 15  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2003:133100  CAPLUS
DN   138:186385
TI   Inactivated Mycobacterium   ***vaccae***   and antigens for modulating
     immune responses and treating autoimmune disease, allergy and graft
     rejection
IN   Watson, James D.;   ***Tan, Paul L. J.***  ; Abernethy, Nevin
PA   Genesis Research and Development Corporation Limited, N. Z.
SO   PCT Int. Appl., 136 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 2003013595      A1   20030220       WO 2002-NZ135    20020726
         W:  AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES,
             FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
             KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
             MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK,
             SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW,
             AM, AZ, BY, KG
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
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PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2003147861 A1 20030807 US 2002-205979 20020725

PRAI US 2001-308446P P 20010726

AB Methods and compns. for the modification of immune response by modulating the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. The components include inactivated Mycobacterium ***vaccae*** ; delipidated or deglycolipidated Mycobacterium ***vaccae*** ; acid or alk. hydrolysis-treated delipidated or deglycolipidated Mycobacterium ***vaccae*** ; or Mycobacterium ***vaccae*** antigens GV-1/70, GV-1/83, GV-3, GV-4P, GV-5, GV-5P, GV--7, GV-9, GV-13, GV-14, GV-22B, GV-23, GV-24B, GV-27, GV-27A, GV-27B, GV-29, GV-33, GV-35, GV-38AP, GV-38BP, GV-40P, GV-41B, GV-42, GV-44 and GV-45. The unwanted immune response or disorders are autoimmune diseases, allergic disorders and graft rejection.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 15 USPATFULL on STN

AN 2003:213224 USPATFULL

TI Compounds and methods for the modulation of immune responses

IN Watson, James D., Auckland, NEW ZEALAND
Tan, Paul L.J. , Bondi Junction, AUSTRALIA
Abernethy, Nevin, Auckland, NEW ZEALAND

PA Genesis Research and Development Corporation Limited, Auckland, NEW ZEALAND (non-U.S. corporation)

PI US 2003147861 A1 20030807

AI US 2002-205979 A1 20020725 (10)

PRAI US 2001-308446P 20010726 (60)

DT Utility

FS APPLICATION

LREP SPECKMAN LAW GROUP, 1501 WESTERN AVE, SUITE 100, SEATTLE, WA, 98101

CLMN Number of Claims: 14

ECL Exemplary Claim: 1

DRWN 29 Drawing Page(s)

LN.CNT 3933

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the modification of immune response by modulating of the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. Such compositions comprise components derived from Mycobacteria, such as Mycobacterium ***vaccae*** .

L4 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

AN 2003:346626 BIOSIS

DN PREV200300346626

TI A genome-based functional screening approach to vaccine development that combines in vitro assays and DNA immunization.

AU Delcayre, Alain [Reprint Author]; Peake, John S.; White, Damian J.; Yuan, Shining; McDonald, Megan K.; Liang, Andrew; ***Tan, Paul L.*** ; Watson, James D.

CS Anosys Inc., 1014 Hamilton Court, Menlo Park, CA, 94025, USA
adelcayre@anosys.com

SO Vaccine, (4 July 2003) Vol. 21, No. 23, pp. 3259-3264. print.
ISSN: 0264-410X (ISSN print).

DT Article

LA English

ED Entered STN: 30 Jul 2003
Last Updated on STN: 30 Jul 2003

AB A two-step screening strategy was developed to identify strong immunogenic polypeptides with putative vaccine andlor adjuvant activity. In the first

step, a mycobacterial genomic DNA library was screened in vitro to identify plasmids encoding polypeptides that stimulate splenocytes from mycobacteria-immunized mice and T cells from PPD-positive healthy donors to produce interferon-gamma. In the second step, plasmids were selected for their ability to induce protective immunity in a mouse model of tuberculosis following DNA immunization. The potential of this approach is illustrated by the identification of a panel of immunogenic polypeptides that may be used to engineer a new generation of vaccines.

L4 ANSWER 5 OF 15 MEDLINE on STN
AN 2004000800 IN-PROCESS
DN PubMed ID: 14629655
TI Pilot study of the safety and effect of intranasal delipidated acid-treated Mycobacterium ***vaccae*** in adult asthma.
AU Shirtcliffe Philippa M; Goldkorn Alexandra; Weatherall Mark; ***Tan Paul*** *** L J*** ; Beasley Richard
CS Wellington Asthma Research Group, Department of Medicine, Wellington School of Medicine, Wellington South, New Zealand.
SO Respirology (Carlton, Vic.), (2003 Dec) 8 (4) 497-503.
Journal code: 9616368. ISSN: 1323-7799.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20040106
Last Updated on STN: 20040108
AB OBJECTIVE: There is epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of atopy and/or asthma. Delipidated, deglycolipidated and arabinogalactan-depleted autoclaved Mycobacterium ***vaccae*** (delipidated acid-treated M. ***vaccae***) has been shown to suppress allergen-induced airway eosinophilia in mice. METHODOLOGY: Thirty-seven adults with stable moderately severe asthma who were skin prick test-positive to house dust mite were randomized to receive two doses 2 weeks apart of delipidated acid-treated M. ***vaccae*** (first dose 0.4 mg and second dose 0.8 mg) or phosphate buffered saline, given as drops intranasally. Safety, tolerability and markers of asthma severity (including peak flow, FEV1, major and minor exacerbations, symptom scores and beta-agonist use), and nasal symptom scores, blood eosinophil and IgE levels were monitored for 8 weeks. RESULTS: Delipidated acid-treated M. ***vaccae*** was safe and well tolerated although there was an occasional mild local reaction. There were no statistically significant differences between the treatment group and placebo for any of the outcome variables. CONCLUSIONS: There is a requirement to elucidate the reasons why mycobacterial-based vaccines have not shown equivalent efficacy in human trials compared with animal models. The role of factors such as duration of disease, route of administration and the active component of mycobacteria need to be addressed.

L4 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:423888 BIOSIS
DN PREV200200423888
TI Compounds and methods for treatment and diagnosis of mycobacterial infections.
AU ***Tan, Paul L.*** [Inventor, Reprint author]; Hiyama, Jun [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corp. Ltd., New Zealand
PI US 6410720 June 25, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 25, 2002) Vol. 1259, No. 4. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent

LA English
ED Entered STN: 7 Aug 2002
Last Updated on STN: 7 Aug 2002
AB The present invention provides polypeptides comprising an immunogenic portion of a M. ***vaccae*** soluble protein and DNA molecules encoding such polypeptides, together with methods for their use in the diagnosis and treatment of mycobacterial infection. Methods for enhancing the immune response to an antigen including administration of M. ***vaccae*** culture filtrate or delipidated M. ***vaccae*** cells are also provided.

L4 ANSWER 7 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:401976 BIOSIS
DN PREV200200401976
TI Compounds and methods for treatment and diagnosis of mycobacterial infections.
AU ***Tan, Paul L.*** [Inventor, Reprint author]; Visser, Elizabeth [Inventor]; Prestidge, Ross [Inventor]; Watson, James D. [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corporation Limited, New Zealand
PI US 6406704 June 18, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 18, 2002) Vol. 1259, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 24 Jul 2002
Last Updated on STN: 24 Jul 2002
AB The present invention provides polypeptides comprising an immunogenic portion of a M. ***vaccae*** protein and DNA molecules encoding such polypeptides, together with methods for their use in the diagnosis and treatment of mycobacterial infection. Methods for enhancing the immune response to an antigen including administration of M. ***vaccae*** culture filtrate, delipidated M. ***vaccae*** cells or delipidated and deglycolipidated M. ***vaccae*** cells are also provided.

L4 ANSWER 8 OF 15 USPATFULL on STN
AN 2002:343542 USPATFULL
TI Methods and compounds for the treatment of immunologically - mediated diseases of the respiratory system using mycobacterium ***vaccae***
IN Watson, James D., Auckland, NEW ZEALAND
Tan, Paul L.J. , Auckland, NEW ZEALAND
PI US 2002197265 A1 20021226
AI US 2002-51643 A1 20020118 (10)
RLI Continuation of Ser. No. US 1998-156181, filed on 17 Sep 1998, PENDING Continuation-in-part of Ser. No. US 1997-996624, filed on 23 Dec 1997, ABANDONED
DT Utility
FS APPLICATION
LREP Janet Sleath, SPECKMAN LAW GROUP, Suite 100, 1501 Western Avenue, Seattle, WA, 98101
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 10 Drawing Page(s)
LN.CNT 6136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment by immunotherapy of lung immune disorders, including infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully

employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such components.

L4 ANSWER 9 OF 15 USPATFULL on STN
AN 2002:39664 USPATFULL
TI Methods and compounds for the treatment of immunologically-mediated diseases using mycobacterium ***vaccae***
IN Watson, James D., Auckland, NEW ZEALAND
Tan, Paul L. J. , Auckland, NEW ZEALAND
Prestidge, Ross, Auckland, NEW ZEALAND
PA Genesis Research & Development Corporation Limited, NEW ZEALAND (non-U.S. corporation)
PI US 6350457 B1 20020226
AI US 1999-449013 19991124 (9)
PRAI US 1999-137112P 19990602 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P
LREP Sleath, Janet, Speckman, Ann W.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1305
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a composition comprising derivatives of delipidated and deglycolipidated M ***vaccae*** cells.

L4 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
AN 2002:113776 BIOSIS
DN PREV200200113776
TI Methods for the treatment of immunologically-mediated skin disorders.
AU Watson, James D. [Inventor, Reprint author]; ***Tan, Paul L. J.*** [Inventor]; Prestidge, Ross [Inventor]
CS Auckland, New Zealand
ASSIGNEE: Genesis Research and Development Corp. Ltd., Parnell, New Zealand
PI US 6328978 December 11, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 11, 2001) Vol. 1253, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 30 Jan 2002
Last Updated on STN: 26 Feb 2002
AB Methods for the treatment of skin disorders, including psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata and skin cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such compositions.

L4 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:320245 BIOSIS

DN PREV200100320245
TI The effect of delipidated deglycolipidated (DDMV) and heat-killed Mycobacterium ***vaccae*** in asthma.
AU Shirtcliffe, Philippa M. [Reprint author]; Easthope, Stephanie E.; Cheng, Soo; Weatherall, Mark; ***Tan, Paul L. J.*** ; Le Gros, Graham; Beasley, Richard
CS Wellington Asthma Research Group, Department of Medicine, Wellington School of Medicine, Wellington South, New Zealand
pip@wnmeds.ac.nz
SO American Journal of Respiratory and Critical Care Medicine, (May, 2001) Vol. 163, No. 6, pp. 1410-1414. print.
ISSN: 1073-449X.
DT Article
LA English
ED Entered STN: 4 Jul 2001
Last Updated on STN: 19 Feb 2002
AB Experimental and epidemiological evidence supports the hypothesis that exposure to mycobacteria has the potential to suppress the development of asthma and/or atopy and there are reports in the Chinese medical literature of repeated vaccination with inactivated BCG being effective in the management of asthma. Forty-three patients with stable moderately severe asthma who were skin prick test positive to house dust mite were randomized to receive two intradermal injections of either phosphate-buffered saline (placebo), heat-killed Mycobacterium ***vaccae*** (0.5 mg), or delipidated deglycolipidated Mycobacterium ***vaccae*** (DDMV) (0.05 mg). Markers of asthma severity were measured for 3 mo and blood eosinophil, IgE levels, and the T cell proliferative and cytokine responses were monitored. There were no significant differences between either treatment group and the placebo group for any of the outcome variables. There was also no difference between the treatment groups and placebo for eosinophil, IgE levels, or the T cell proliferative and cytokine response. The results indicate no effect of low dose intradermal DDMV or M. ***vaccae*** on asthma severity in patients with established asthma.

L4 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:880993 CAPLUS
DN 134:41096
TI Methods and compounds for the treatment of immunologically-mediated diseases using Mycobacterium ***vaccae***
IN Watson, James D.; ***Tan, Paul L. J.*** ; Prestidge, Ross L.
PA Genesis Research & Development Corporation Limited, N. Z.
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000074715	A1	20001214	WO 2000-NZ85	20000601
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6350457	B1	20020226	US 1999-449013	19991124
EP 1181051	A1	20020227	EP 2000-937399	20000601
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, MC, PT, IE, SI, LT, LV, FI, RO				

	BR 2000011239	A	20020402	BR 2000-11239	20000601
	JP 2003501400	T2	20030114	JP 2001-501249	20000601
PRAI	US 1999-137112P	P	19990602		
	US 1999-449013	A	19991124		
	WO 2000-NZ85	W	20000601		

AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as (M. tuberculosis) or (M. avium), sarcoidosis, asthma, allergic rhinitis and lung cancers are provided, such methods comprising administering a compn. comprising at least one deriv. of delipidated and deglycolipidated (M. ***vaccae***) cells.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:140561 BIOSIS
DN PREV200000140561
TI Improvement in psoriasis after intradermal administration of heat-killed Mycobacterium ***vaccae*** .
AU Balagon, Maria V.; Walsh, Douglas S. [Reprint author]; ***Tan, Paul*** *** L.*** ; Cellona, Roland V.; Abalos, Rodolfo M.; Tan, Esterlina V.; Fajardo, Tranquilino T., Jr.; Watson, James D.; Walsh, Gerald P.
CS Armed Forces Research Institute of Medical Sciences (AFRIMS), APO, AP, 96546-5000, USA
SO International Journal of Dermatology, (Jan., 2000) Vol. 39, No. 1, pp. 51-58. print.
CODEN: IJDEBB. ISSN: 0011-9059.
DT Article
LA English
ED Entered STN: 19 Apr 2000
Last Updated on STN: 4 Jan 2002
AB Background: New treatments for psoriasis are being developed, but many are associated with limited efficacy, side-effects, or rapid recurrence after discontinuation. Thus, the aim of new agents is to induce longer term remissions with fewer side-effects. Preliminary studies have shown that Mycobacterium ***vaccae*** , a nonpathogenic organism prepared as a heat-killed suspension, may induce periods of remission in some psoriasis patients when administered intradermally. Methods: To further assess the efficacy and tolerability of M. ***vaccae*** in patients with moderate to severe psoriasis (psoriasis area and severity index (PASI) of 12-35), we conducted an open label study whereby 24 patients received two intradermal inoculations of M. ***vaccae*** in lesion-free deltoid skin, separated by a period of 3 weeks. Results: Twelve weeks after starting treatment, 14 of 24 patients (58%) showed marked improvement in the PASI score (greater than 50% reduction), two had moderate improvement (25-50% reduction), six were unchanged (< 25% reduction), and two had worsened (> 5% increase). By 24 weeks, 11 of 22 patients continued to show greater than 50% improvement. Five patients had complete clearance of skin lesions that lasted for at least 6 months. Conclusions: Intradermal administration of heat-killed M. ***vaccae*** suspension was well tolerated and induced clinically significant improvement in a majority of psoriasis patients in this cohort. Placebo-controlled testing to further define the efficacy of this treatment is warranted.

L4 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 2000:283534 BIOSIS
DN PREV200000283534
TI Methods and compounds for the treatment of immunologically-mediated psoriasis.
AU Watson, James D. [Inventor, Reprint author]; ***Tan, Paul L. J.*** [Inventor]
CS Auckland, New Zealand

ASSIGNEE: Genesis Research and Development Corp., Auckland, New Zealand
PI US 5968524 October 19, 1999
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 19, 1999) Vol. 1227, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002
AB Methods for the treatment of skin disorders, including psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata and skin cancers are provided, such methods comprising administering multiple doses of a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, delipidated and deglycolipidated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such compositions.

L4 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 1997:515428 BIOSIS
DN PREV199799814631
TI Immunization with heat-killed Mycobacterium ***vaccae*** stimulates CD8+ cytotoxic T cells specific for macrophages infected with Mycobacterium tuberculosis.
AU Skinner, Margot A.; Yuan, Shining; Prestidge, Ross; Chuk, David; Watson, James D.; ***Tan, Paul L. J.*** [Reprint author]
CS Genesis Res. Dev. Corp. Ltd., PO Box 50, Auckland, New Zealand
SO Infection and Immunity, (1997) Vol. 65, No. 11, pp. 4525-4530.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 10 Dec 1997
Last Updated on STN: 10 Dec 1997
AB Immune responses to Mycobacterium tuberculosis are analyzed in mice which have been immunized with Mycobacterium ***vaccae*** to examine novel ways of altering protective immunity against M. tuberculosis. The spleen cells of mice immunized with M. ***vaccae*** proliferate and secrete gamma interferon (IFN-gamma) in response to challenge with live M. tuberculosis in vitro. Immunization with M. ***vaccae*** results in the generation of CD8+ T cells which kill syngeneic macrophages infected with M. tuberculosis. These effector cytotoxic T cells (CTL) are detectable in the spleen at 2 weeks after immunization with M. ***vaccae*** but cannot be found in splenocytes 3 to 6 weeks postimmunization. However, M. tuberculosis-specific CTL are revealed following restimulation in vitro with heat-killed M. ***vaccae*** or M. tuberculosis, consistent with the activation of memory cells. These CD8+ T cells secrete IFN-gamma and enhance the production of interleukin 12 when cocultured with M. tuberculosis-infected macrophages. It is suggested that CD8+ T cells with a cytokine secretion profile of the Tc1 class may themselves maintain the dominance of a Th1-type cytokine response following immunization with M. ***vaccae*** . Heat-killed M. ***vaccae*** deserves attention as an alternative to attenuated live mycobacterial vaccines.

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=> s vacca? and (delipidat? or deglycolipidat?)
L5          58 VACCA? AND (DELIPIDAT? OR DEGLYCOLIPIDAT?)
=> dup rem l5
PROCESSING COMPLETED FOR L5
L6           32 DUP REM L5 (26 DUPLICATES REMOVED)
=> s l6 and asthm?
L7          10 L6 AND ASTHM?
=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 10 ANSWERS - CONTINUE? Y/(N):y
L7   ANSWER 1 OF 10  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN   2001:320245  BIOSIS
DN   PREV200100320245
TI   The effect of   ***delipidated***     ***deglycolipidated***   (DDMV) and
     heat-killed Mycobacterium   ***vaccae***   in   ***asthma***   .
AU   Shirtcliffe, Philippa M. [Reprint author]; Easthope, Stephanie E.; Cheng,
     Soo; Weatherall, Mark; Tan, Paul L. J.; Le Gros, Graham; Beasley, Richard
CS   Wellington Asthma Research Group, Department of Medicine, Wellington
     School of Medicine, Wellington South, New Zealand
     pip@wnmeds.ac.nz
SO   American Journal of Respiratory and Critical Care Medicine, (May, 2001)
     Vol. 163, No. 6, pp. 1410-1414. print.
     ISSN: 1073-449X.
DT   Article
LA   English
ED   Entered STN: 4 Jul 2001
     Last Updated on STN: 19 Feb 2002
AB   Experimental and epidemiological evidence supports the hypothesis that
     exposure to mycobacteria has the potential to suppress the development of
       ***asthma***   and/or atopy and there are reports in the Chinese medical
     literature of repeated vaccination with inactivated BCG being effective in
     the management of   ***asthma***   . Forty-three patients with stable
     moderately severe   ***asthma***   who were skin prick test positive to
     house dust mite were randomized to receive two intradermal injections of
     either phosphate-buffered saline (placebo), heat-killed Mycobacterium
       ***vaccae***   (0.5 mg), or   ***delipidated***     ***deglycolipidated***
     Mycobacterium   ***vaccae***   (DDMV) (0.05 mg). Markers of
       ***asthma***   severity were measured for 3 mo and blood eosinophil, IgE
     levels, and the T cell proliferative and cytokine responses were
     monitored. There were no significant differences between either treatment
     group and the placebo group for any of the outcome variables. There was
     also no difference between the treatment groups and placebo for
     eosinophil, IgE levels, or the T cell proliferative and cytokine response.
     The results indicate no effect of low dose intradermal DDMV or M.
       ***vaccae***   on   ***asthma***   severity in patients with established
       ***asthma***   .
TI   The effect of   ***delipidated***     ***deglycolipidated***   (DDMV) and
     heat-killed Mycobacterium   ***vaccae***   in   ***asthma***   .
AB   Experimental and epidemiological evidence supports the hypothesis that
     exposure to mycobacteria has the potential to suppress the development of
       ***asthma***   and/or atopy and there are reports in the Chinese medical
     literature of repeated vaccination with inactivated BCG being effective in
     the management of   ***asthma***   . Forty-three patients with stable
     moderately severe   ***asthma***   who were skin prick test positive to
     house dust mite were randomized to receive two intradermal injections of
     either phosphate-buffered saline (placebo), heat-killed Mycobacterium
       ***vaccae***   (0.5 mg), or   ***delipidated***     ***deglycolipidated***
     Mycobacterium   ***vaccae***   (DDMV) (0.05 mg). Markers of
       ***asthma***   severity were measured for 3 mo and blood eosinophil, IgE
     levels, and the T cell proliferative and cytokine responses were. . .
     or the T cell proliferative and cytokine response. The results indicate
     no effect of low dose intradermal DDMV or M.   ***vaccae***   on
       ***asthma***   severity in patients with established   ***asthma***   .
IT   . . .
```

Structures, & Systems of Organisms
T cells: blood and lymphatics, immune system; eosinophils: blood and lymphatics, immune system
IT Diseases
asthma : immune system disease, respiratory system disease, management, severity
Asthma (MeSH)
IT Chemicals & Biochemicals
IgE [immunoglobulin E]; cytokines; ***delipidated*** ***deglycolipidated*** Mycobacterium ***vaccae*** vaccine: immunologic-drug, intradermal administration, vaccine; heat-killed Mycobacterium ***vaccae*** vaccine: immunologic-drug, intradermal administration, vaccine
ORGN . . .
Mammals, Primates, Vertebrates
ORGN Classifier
Mycobacteriaceae 08881
Super Taxa
Mycobacteria; Actinomycetes and Related Organisms; Eubacteria; Bacteria; Microorganisms
Organism Name
Mycobacterium ***vaccae*** : ***delipidated*** ***deglycolipidated*** , heat-killed
Taxa Notes
Bacteria, Eubacteria, Microorganisms

L7 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:880993 CAPLUS
DN 134:41096
TI Methods and compounds for the treatment of immunologically-mediated diseases using Mycobacterium ***vaccae***
IN Watson, James D.; Tan, Paul L. J.; Prestidge, Ross L.
PA Genesis Research & Development Corporation Limited, N. Z.
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000074715	A1	20001214	WO 2000-NZ85	20000601
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6350457	B1	20020226	US 1999-449013	19991124
	EP 1181051	A1	20020227	EP 2000-937399	20000601
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, MC, PT, IE, SI, LT, LV, FI, RO				
	BR 2000011239	A	20020402	BR 2000-11239	20000601
	JP 2003501400	T2	20030114	JP 2001-501249	20000601
PRAI	US 1999-137112P	P	19990602		
	US 1999-449013	A	19991124		
	WO 2000-NZ85	W	20000601		

AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as (M. tuberculosis) or (M. avium), sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising

administering a compn. comprising at least one deriv. of ***delipidated*** and ***deglycolipidated*** (M. ***vaccae***) cells.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Methods and compounds for the treatment of immunologically-mediated diseases using Mycobacterium ***vaccae***

AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as (M. tuberculosis) or (M. avium), sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising administering a compn. comprising at least one deriv. of ***delipidated*** and ***deglycolipidated*** (M. ***vaccae***) cells.

ST ***delipidated*** ***deglycolipidated*** Mycobacterium ***vaccae*** immunol disease

IT Cell activation
(T cell; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT T cell (lymphocyte)
(activation; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Immunostimulants
(adjuvants; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Hydrolysis
(alk. or acid; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Nose
(allergic rhinitis; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Dermatitis
(atopic; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Infection
(bacterial; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Hydrolysis
(base; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Aerosols
Asthma
Atherosclerosis
Eczema
Eosinophilia
Hypercholesterolemia
Infection
Lung, neoplasm
Mycobacterium
Mycobacterium avium
Mycobacterium smegmatis
Mycobacterium tuberculosis
Mycobacterium ***vaccae***
Neoplasm
Sarcoidosis

Vaccines
(***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Interleukin 10
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Carbohydrates, processes
Glycolipids
Lipids, processes
Mycolic acids
RL: REM (Removal or disposal); PROC (Process)
(***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Respiratory tract
(disease; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Acids, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(hydrolysis; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Drug delivery systems
(injections, s.c.; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Diabetes mellitus
(insulin-dependent; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Drug delivery systems
(intradermal; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Lymphocyte
(natural killer cell, activation; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Epithelium
(repairing; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT Drug delivery systems
(transdermal; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT T cell (lymphocyte)
(.alpha..beta. and .gamma..delta.; ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT 7664-39-3, Anhydrous hydrofluoric acid, biological studies 13444-71-8, Periodic acid 39450-01-6
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** for treatment of immunol.-mediated diseases)

IT 59-23-4, Galactose, processes 3416-24-8, Glucosamine 9036-66-2, Arabinogalactan
RL: REM (Removal or disposal); PROC (Process)
(***delipidated*** and ***deglycolipidated*** Mycobacterium

vaccae for treatment of immunol.-mediated diseases)

L7 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:421786 CAPLUS
DN 131:56388
TI Proteins of Mycobacterium ***vaccae*** and the genes encoding them and their use in the diagnosis and treatment of mycobacterial disease
IN Tan, Paul; Watson, James; Visser, Elizabeth S.; Skinner, Margot A.; Prestidge, Ross L.
PA Genesis Research & Development Corporation Limited, N. Z.
SO PCT Int. Appl., 243 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 8

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9932634	A2	19990701	WO 1998-NZ189	19981223
	WO 9932634	A3	19991202		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 5968524	A	19991019	US 1997-997080	19971223
	US 5985287	A	19991116	US 1997-997362	19971223
	US 6160093	A	20001212	US 1998-95855	19980611
	US 6406704	B1	20020618	US 1998-205426	19981204
	CA 2315539	AA	19990701	CA 1998-2315539	19981223
	AU 9918936	A1	19990712	AU 1999-18936	19981223
	AU 746311	B2	20020418		
	BR 9814432	A	20001010	BR 1998-14432	19981223
	EP 1044273	A2	20001018	EP 1998-963665	19981223
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002514385	T2	20020521	JP 2000-525553	19981223
	NZ 505834	A	20021220	NZ 1998-505834	19981223
	NO 2000003261	A	20000822	NO 2000-3261	20000622
	US 2002197265	A1	20021226	US 2002-51643	20020118
PRAI	US 1997-996624	A	19971223		
	US 1997-997080	A	19971223		
	US 1997-997362	A	19971223		
	US 1998-95855	A	19980611		
	US 1998-156181	A	19980917		
	US 1998-205426	A	19981204		
	US 1996-705347	A2	19960829		
	US 1997-873970	A2	19970612		
	WO 1998-NZ189	W	19981223		

AB Antigenic and adjuvant proteins of the non-pathogenic Mycobacterium ***vaccae*** that may be of use in the diagnosis, treatment, and prophylaxis of a range of diseases with a mycobacterial etiol. are described. These proteins appear to stimulate Th1 cell function. Diseases that may be treated include infection with Mycobacterium tuberculosis and M. avium, ***asthma*** , sarcoidosis, lung cancer, and a no. of skin diseases. Methods for increasing the immune response to an antigen including administration of M. ***vaccae*** culture filtrate, ***delipidated*** M. ***vaccae*** cells, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells depleted of mycolic acids, and ***delipidated*** and ***deglycolipidated*** M.

vaccae cells depleted of mycolic acids and arabinogalactan are also provided. Vaccination of mice and green monkeys with culture filtrates of M. ***vaccae*** was found to offer significant protection against subsequent challenge with M. tuberculosis. Heat-killed M. ***vaccae*** was also able to ameliorate the effects of psoriasis in humans with rebuilding of normal skin structure. These effects in part appear to be due to stimulation of interleukin 12 prodn. by macrophages and, to a lesser extent, interferon .gamma. prodn. by NK cells. Proteins of culture filtrates were purified by std. methods and partial amino acid sequences used to design primers for cloning of genes via PCR.

TI Proteins of Mycobacterium ***vaccae*** and the genes encoding them and their use in the diagnosis and treatment of mycobacterial disease

AB Antigenic and adjuvant proteins of the non-pathogenic Mycobacterium ***vaccae*** that may be of use in the diagnosis, treatment, and prophylaxis of a range of diseases with a mycobacterial etiol. are described. These proteins appear to stimulate Th1 cell function. Diseases that may be treated include infection with Mycobacterium tuberculosis and M. avium, ***asthma*** , sarcoidosis, lung cancer, and a no. of skin diseases. Methods for increasing the immune response to an antigen including administration of M. ***vaccae*** culture filtrate, ***delipidated*** M. ***vaccae*** cells, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells depleted of mycolic acids, and ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells depleted of mycolic acids and arabinogalactan are also provided. Vaccination of mice and green monkeys with culture filtrates of M. ***vaccae*** was found to offer significant protection against subsequent challenge with M. tuberculosis. Heat-killed M. ***vaccae*** was also able to ameliorate the effects of psoriasis in humans with rebuilding of normal skin structure. These effects in part appear to be due to stimulation of interleukin 12 prodn. by macrophages and, to a lesser extent, interferon .gamma. prodn. by NK cells. Proteins of culture filtrates were purified by std. methods and partial amino acid sequences used to design primers for cloning of genes via PCR.

ST Mycobacterium vaccine antigen protein gene cloning; adjuvant protein Mycobacterium ***vaccae*** ; interleukin 12 synthesis Mycobacterium protein inducer

IT Cell activation
(B cell, Mycobacterium ***vaccae*** proteins stimulating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Glycolipids
Lipids, biological studies
Mycolic acids
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Mycobacterium ***vaccae*** free of, in vaccines; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Antigen-presenting cell
(Mycobacterium ***vaccae*** proteins activating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Macrophage
(Mycobacterium ***vaccae*** stimulation of interleukin 12 prodn. by; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Interleukin 12
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(Mycobacterium ***vaccae*** stimulation of macrophage prodn. of; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Cell activation

Cell proliferation
(T cell, Mycobacterium ***vaccae*** proteins stimulating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT T cell (lymphocyte)
(activation, Mycobacterium ***vaccae*** proteins stimulating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Immunostimulants
(adjuvants, proteins of Mycobacterium ***vaccae*** as; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT CD8-positive T cell
(cytotoxic to Mycobacterium tuberculosis-infected macrophages, M. ***vaccae*** induction of; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Cerebrospinal fluid
Saliva
(diagnostic detection of proteins of Mycobacterium in; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Immunoassay
(for proteins of Mycobacterium ***vaccae*** ; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Blood analysis
Urine analysis
(for proteins of Mycobacterium; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Antibodies
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(monoclonal, to proteins of Mycobacterium ***vaccae*** ; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Cell activation
(monocyte, Mycobacterium ***vaccae*** proteins stimulating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Lymphocyte
(natural killer cell, Mycobacterium ***vaccae*** stimulation of interferon .gamma. prodn. by; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Antigens
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(of Mycobacterium ***vaccae*** ; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT T cell (lymphocyte)
(proliferation, Mycobacterium ***vaccae*** proteins stimulating; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Mycobacterium
Mycobacterium ***vaccae***
(proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Antibodies
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(to proteins of Mycobacterium ***vaccae*** ; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Mycobacterium avium
(treatment and prophylaxis of infection by; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT ***Asthma***
Lung, neoplasm
Psoriasis
Sarcoidosis
Tuberculosis
(treatment and prophylaxis of; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Skin, disease
(treatment and prophylaxis; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT Interferons
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(.gamma., Mycobacterium ***vaccae*** stimulation of NK cell prodn. of; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT 9036-66-2D, Arabinogalactan, polymers, derivs.
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Mycobacterium ***vaccae*** free of, in vaccines; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT 204785-82-0 228105-87-1, Antigen GVc-13 (Mycobacterium ***vaccae***)
228106-68-1, Antigen GV-22B (Mycobacterium ***vaccae***)
228107-02-6, Antigen GV-1/83 (Mycobacterium ***vaccae***)
228107-33-3, Antigen GV-1/70 (Mycobacterium ***vaccae***)
228107-35-5, Antigen GVs-9 (Mycobacterium ***vaccae***) 228107-37-7
228107-40-2, Antigen GV-5P (Mycobacterium ***vaccae***) 228107-42-4, Antigen GV-27 (Mycobacterium ***vaccae***) 228107-44-6, Antigen GV-27B (Mycobacterium ***vaccae***) 228107-47-9 228107-48-0
228107-53-7, Antigen GV-24B (Mycobacterium ***vaccae***) 228107-57-1
228107-59-3 228107-62-8, Antigen GV-38A (Mycobacterium ***vaccae***)
228107-65-1, Antigen GV-38B (Mycobacterium ***vaccae***)
228107-68-4, Antigen GV-41 (Mycobacterium ***vaccae***) 228107-70-8, Antigen GV-42 (Mycobacterium ***vaccae***) 228107-73-1, Antigen GV-44 (Mycobacterium ***vaccae***) 228107-74-2 228107-76-4
228107-79-7, Antigen GV-33 (Mycobacterium ***vaccae***) 228107-83-3, Antigen GVc-13 (Mycobacterium ***vaccae***) 228107-96-8, Antigen GVs-9 (Mycobacterium ***vaccae***) 228107-99-1, Antigen GV-29 (Mycobacterium ***vaccae***) 228108-01-8, Antigen GV-45 (Mycobacterium ***vaccae***) 228108-08-5, Antigen GV-41B (Mycobacterium ***vaccae***) 228108-12-1, Antigen GV-44 (Mycobacterium ***vaccae***) 228108-14-3 228111-24-8
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

IT 228107-21-9 228107-30-0 228107-31-1 228107-32-2 228107-34-4
228107-36-6 228107-39-9 228107-41-3 228107-43-5 228107-45-7
228107-46-8 228107-49-1 228107-52-6 228107-56-0 228107-58-2
228107-60-6 228107-61-7 228107-66-2 228107-67-3 228107-71-9
228107-72-0 228107-75-3 228107-78-6 228107-98-0 228108-00-7
228108-02-9 228108-11-0 228108-13-2

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; proteins of Mycobacterium ***vaccae*** and genes encoding them and their use in diagnosis and treatment of mycobacterial disease)

L7 ANSWER 4 OF 10 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

AN 2004002957 EMBASE

TI Pilot study of the safety and effect of intranasal ***delipidated*** acid-treated Mycobacterium ***vaccae*** in adult ***asthma*** .

AU Shirtcliffe P.M.; Goldkorn A.; Weatherall M.; Tan P.L.J.; Beasley R.

CS R. Beasley, Med. Res. Institute of New Zealand, PO Box 10-055, Wellington, New Zealand. richard.beasley@mrinz.ac.nz

SO Respirology, (2003) 8/4 (497-503).
Refs: 19
ISSN: 1323-7799 CODEN: RSPIFB

CY Australia

DT Journal; Article

FS 015 Chest Diseases, Thoracic Surgery and Tuberculosis
017 Public Health, Social Medicine and Epidemiology
037 Drug Literature Index
038 Adverse Reactions Titles

LA English

SL English

AB Objective: There is epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of atopy and/or ***asthma*** . ***Delipidated*** , ***deglycolipidated*** and arabinogalactan-depleted autoclaved Mycobacterium ***vaccae*** (***delipidated*** acid-treated M. ***vaccae***) has been shown to suppress allergen-induced airway eosinophilia in mice. Methodology: Thirty-seven adults with stable moderately severe ***asthma*** who were skin prick test-positive to house dust mite were randomized to receive two doses 2 weeks apart of ***delipidated*** acid-treated M. ***vaccae*** (first dose 0.4 mg and second dose 0.8 mg) or phosphate buffered saline, given as drops intranasally. Safety, tolerability and markers of ***asthma*** severity (including peak flow, FEV (1), major and minor exacerbations, symptom scores and beta-agonist use), and nasal symptom scores, blood eosinophil and IgE levels were monitored for 8 weeks. Results: ***Delipidated*** acid-treated M. ***vaccae*** was safe and well tolerated although there was an occasional mild local reaction. There were no statistically significant differences between the treatment group and placebo for any of the outcome variables. Conclusions: There is a requirement to elucidate the reasons why mycobacterial-based vaccines have not shown equivalent efficacy in human trials compared with animal models. The role of factors such as duration of disease, route of administration and the active component of mycobacteria need to be addressed.

TI Pilot study of the safety and effect of intranasal ***delipidated*** acid-treated Mycobacterium ***vaccae*** in adult ***asthma*** .

AB . . . There is epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of atopy and/or ***asthma*** . ***Delipidated*** , ***deglycolipidated*** and arabinogalactan-depleted autoclaved Mycobacterium ***vaccae*** (***delipidated*** acid-treated M. ***vaccae***) has been shown to suppress allergen-induced airway eosinophilia in mice. Methodology: Thirty-seven adults with stable moderately severe ***asthma*** who were skin prick test-positive to house dust mite were randomized to receive two doses 2 weeks apart of ***delipidated*** acid-treated M. ***vaccae*** (first dose 0.4 mg and second dose 0.8 mg) or phosphate buffered saline, given as drops intranasally. Safety, tolerability and markers of ***asthma*** severity (including peak flow, FEV (1), major and minor exacerbations, symptom scores and beta-agonist use), and nasal

symptom scores, blood eosinophil and IgE levels were monitored for 8 weeks. Results: ***Delipidated*** acid-treated M. ***vaccae*** was safe and well tolerated although there was an occasional mild local reaction. There were no statistically significant differences between. .
.

CT Medical Descriptors:
****Mycobacterium vaccae***
****asthma: DT, drug therapy***
****asthma: EP, epidemiology***
pilot study
drug safety
drug efficacy
atopy
autoclave
eosinophilia
disease severity
skin test
prick test
Dermatophagoides
randomization
drug tolerability
treatment outcome
peak expiratory flow
forced expiratory volume
eosinophil
vaccination reaction: SI, side effect
disease course
vaccination
disease exacerbation
human
clinical article
clinical trial
randomized controlled trial
double blind procedure
controlled study
adolescent
adult
article
priority journal
****Mycobacterium vaccae vaccine: AE, adverse drug reaction***
****Mycobacterium vaccae vaccine: CT, clinical trial***
****Mycobacterium vaccae vaccine: DO, drug dose***
****Mycobacterium vaccae vaccine: DT, drug therapy***
****Mycobacterium vaccae vaccine: PD, pharmacology***
****Mycobacterium vaccae vaccine: NA, intranasal drug administration***
*vaccine: AE, adverse drug reaction
*vaccine: CT, clinical trial
*vaccine: DO, drug dose
*vaccine: DT, drug therapy
*vaccine: PD,. . .

L7 ANSWER 5 OF 10 USPATFULL on STN
AN 2003:213224 USPATFULL
TI Compounds and methods for the modulation of immune responses
IN Watson, James D., Auckland, NEW ZEALAND
Tan, Paul L.J., Bondi Junction, AUSTRALIA
Abernethy, Nevin, Auckland, NEW ZEALAND
PA Genesis Research and Development Corporation Limited, Auckland, NEW ZEALAND (non-U.S. corporation)
PI US 2003147861 A1 20030807
AI US 2002-205979 A1 20020725 (10)
PRAI US 2001-308446P 20010726 (60)
DT Utility

FS APPLICATION
LREP SPECKMAN LAW GROUP, 1501 WESTERN AVE, SUITE 100, SEATTLE, WA, 98101
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 29 Drawing Page(s)
LN.CNT 3933
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the modification of immune response by modulating of the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. Such compositions comprise components derived from Mycobacteria, such as Mycobacterium ***vaccae*** .

AB . . . disorders characterized by the presence of an unwanted immune response. Such compositions comprise components derived from Mycobacteria, such as Mycobacterium ***vaccae*** .

SUMM . . . the inventive methods comprise administering a composition, wherein the composition comprises inactivated mycobacterial cells or a derivative thereof, such as ***delipidated*** and ***deglycolipidated*** mycobacterial cells. In preferred embodiments, the ***delipidated*** and ***deglycolipidated*** cells are prepared from M. ***vaccae*** , M. tuberculosis or M. smegmatis. In further embodiments, the inventive methods comprise administering a composition comprising peptidoglycan.

SUMM [0013] In other embodiments, the compositions employed in the inventive methods comprise a derivative of ***delipidated*** and ***deglycolipidated*** mycobacterial cells, the derivative being selected from the group consisting of: ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated by acid hydrolysis; ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated by alkaline hydrolysis; ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated with periodic acid; ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated with Proteinase K; and ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated by anhydrous hydrofluoric acid hydrolysis. In specific embodiments, such derivatives are prepared from M. ***vaccae*** , M. tuberculosis or M. smegmatis. The derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** contain glucosamine in an amount greater than 3.7% of total carbohydrate, preferably greater than 5% total carbohydrate and more preferably. . .

SUMM [0014] In yet another aspect, the compositions disclosed herein comprise an isolated polypeptide derived from Mycobacterium ***vaccae*** or an isolated polynucleotide encoding such a polypeptide, such polypeptides comprising at least an immunogenic portion of an M. ***vaccae*** antigen, or a variant thereof. In specific embodiments, such polypeptides comprise an amino acid sequence selected from the group consisting. . .

DRWD [0016] FIG. 1 illustrates the re-suspension of DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH.

DRWD [0017] FIG. 2 shows the suppression by DD-M. ***vaccae*** (Q1) and the DD-M. ***vaccae*** derivatives Q2 (DD-M. ***vaccae*** -KOH), Q3 (DD-M. ***vaccae*** -acid), Q4 (DD-M. ***vaccae*** -periodate), Q6 (DD-M. ***vaccae*** -KOH-periodate), P5 (DD-M. ***vaccae*** -KOH-acid) and P6 (DD-M. ***vaccae*** -KOH-periodate) of ovalbumin-induced airway eosinophilia in mice vaccinated intranasally with these compounds. Control mice received PBS.

DRWD [0018] FIG. 3 illustrates the effect of immunization with DD-M. ***vaccae*** on airway eosinophilia when administered either one day

prior, at the time of, or one day after challenge with OVA.

DRWD [0019] FIG. 4 shows the stimulation of IL-10 production in THP-1 cells by derivatives of DD-M. ***vaccae*** .

DRWD [0020] FIG. 5 illustrates the effect of immunization with DD-M. ***vaccae*** , DD-M. tuberculosis and DD-M. smegmatis on airway eosinophilia.

DRWD [0021] FIG. 6 illustrates TNF-.alpha. production by human PBMC and non-adherent cells stimulated with DD-M. ***vaccae*** .

DRWD [0022] FIGS. 7A and 7B illustrate IL-10 and IFN-.gamma. production, respectively, by human PBMC and non-adherent cells stimulated with DD-M. ***vaccae*** .

DRWD . . . FIGS. 8A-C illustrate the stimulation of CD69 expression on .alpha..beta.T cells, .gamma..delta.T cells and NK cells, respectively, by the M. ***vaccae*** protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

DRWD [0024] FIGS. 9A-D illustrate the effect of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** and M. ***vaccae*** recombinant proteins on the production of IL-1.beta., TNF-.alpha., IL-12 and IFN-.gamma., respectively, by human PBMC.

DRWD [0025] FIGS. 10A-C illustrate the effects of varying concentrations of the recombinant M. ***vaccae*** proteins GV-23 and GV-45 on the production of IL-1.beta., TNF-.alpha. and IL-12, respectively, by human PBMC.

DRWD [0026] FIGS. 11A-D illustrate the stimulation of IL-1.beta., TNF-.alpha., IL-12 and IFN-.gamma. production, respectively, in human PBMC by the M. ***vaccae*** protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

DRWD [0027] FIGS. 12A-C illustrate the effects of varying concentrations of the recombinant M. ***vaccae*** proteins GV-23 and GV-45 on the expression of CD40, CD80 and CD86, respectively, by dendritic cells.

DRWD [0028] FIG. 13 illustrates the enhancement of dendritic cell mixed lymphocyte reaction by the recombinant M. ***vaccae*** protein GV-23.

DRWD [0030] FIG. 15A-C illustrate the effect of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** (referred to in the Figure as PVAC) and AVAC, respectively, on the expression of genes involved in Notch signaling in. . .

DRWD [0031] FIG. 16 illustrates the effect of intranasal administration of AVAC and DD-M. ***vaccae*** (referred to in the Figure as PVAC) in mice on expression of genes involved in Notch signaling.

DRWD [0033] FIG. 18 shows the production of IL-12p40 by THP-1 cells in response to increasing concentrations of M. ***vaccae*** derivatives.

DRWD [0034] FIG. 19 shows the production of IL-12p40, IL-23p19 and IL-12p35 mRNA in THP-1 cells in response to AVAC, DD-M. ***vaccae*** , heat-killed M. ***vaccae*** and M. ***vaccae*** glycolipids.

DRWD . . . FIGS. 20A-C illustrate the production of IL-12p40 by THP-1 cells cultured with antibodies to Toll-like receptors and either heat-killed M. ***vaccae*** , DD-M. ***vaccae*** or AVAC, respectively.

DRWD . . . FIGS. 21A-C illustrate the production of TNF-alpha by THP-1 cells cultured with antibodies to Toll-like receptors and either heat-killed M. ***vaccae*** , DD-M. ***vaccae*** or LPS, respectively.

DRWD [0037] FIG. 22 shows the production of IL-10 by THP-1 cells cultured with antibodies to Toll-like receptors and heat-killed M. ***vaccae*** .

DETD . . . rheumatoid arthritis, Type I diabetes mellitus, psoriasis, systemic lupus erythematosus and scleroderma. Examples of allergic disorders include atopic dermatitis, eczema, ***asthma*** , allergic rhinitis, contact allergies and hypersensitivities.

DETD . . . shown to up-regulate Th1 responses, while IL-10 has been shown to down-regulate Th2 responses. The inventors have discovered that both ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells (referred to herein as DD-M. ***vaccae***) and ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells further treated by acid hydrolysis (referred to herein as AVAC) have pronounced immunoregulatory effects on both Th2 and Th1 cells. For example, as detailed below, the inventors have demonstrated the efficacy of both DD-M. ***vaccae*** and AVAC in the treatment of ***asthma*** employing a mouse model. These compositions are believed to be effective in the treatment of diseases such as ***asthma*** due to their ability to down-regulate ***asthma*** -inducing Th2 immune responses, as shown by the reduction in total IgE and antigen-specific IgE and IgG1.

DETD [0042] In clinical trials on the effectiveness of DD-M. ***vaccae*** in the treatment psoriasis, local injections of DD-M. ***vaccae*** were observed to lead to clearance of distant skin lesions, demonstrating the involvement of a systemic mechanism of action. No in vitro proliferation in response to DD-M. ***vaccae*** stimulation was observed in peripheral blood mononuclear cells (PBMC) taken from DD-M. ***vaccae*** -treated patients, thereby indicating the lack of a specific T cell response to DD-M. ***vaccae*** . Experimental data is presented, below, in Example 9.

DETD [0043] As described below, DD-M. ***vaccae*** is ingested by cells of the THP-1 human monocytic cell line and stimulates these cells to secrete IL-10 and IL-12. DD-M. ***vaccae*** stimulates blood-derived human dendritic cells to upregulate the expression of CD40, CD80 and CD86 costimulatory molecules in vitro. T cell and NK cells show increased expression of the CD69 activation molecule when exposed to DD-M. ***vaccae*** , and the antigen presenting function of mouse dendritic cells is enhanced when bone marrow derived dendritic cells are pre-tested with DD-M. ***vaccae*** in vitro. Taken together, these results indicate that DD-M. ***vaccae*** modifies the response to endogenous psoriatic antigen by affecting antigen presentation.

DETD [0044] As the clinical effects of DD-M. ***vaccae*** on psoriasis are systemic and distant psoriatic lesions are cleared following local injection of DD-M. ***vaccae*** , it is likely that DD-M. ***vaccae*** is transported to the lymph nodes where it influences APCs and T cells. Alternatively, either APCs or both APCs and regulatory T cells activated by DD-M. ***vaccae*** migrate to lymph nodes and the circulation. The APCs then terminate the generation of pathologic T cells, and T cells. . .

DETD . . . during development are likely to influence the differentiation of T cell subsets during an immune response. The fact that DD-M. ***vaccae*** and its derivatives do not suppress antigen presentation and stimulate cytokine production, indicates that they may be successfully employed to. . .

DETD [0046] As detailed below, the inventors have demonstrated that a derivative of DD-M. ***vaccae*** , namely AVAC, induces production of Notch ligands on antigen presenting cells (APCs). Recognition of an antigen on these up-regulated APCs,. . . T cells, leading to the induction of infectious tolerance to the antigen. The inventors have also demonstrated that AVAC, DD-M. ***vaccae*** , inactivated M. ***vaccae*** and M. ***vaccae*** glycolipids modulate expression of various genes involved in Notch signaling both in vitro and in vivo, as well as genes. . .

DETD . . . wishing to be bound by theory, the inventors believe, based on the experimental results presented below, that interaction of M. ***vaccae*** , DD-M. ***vaccae*** and AVAC with human myelomonocytic THP-1 cells is mediated in part by the specific binding of M. ***vaccae*** -derived cell wall components, principally peptidoglycan, to the extracellular domain of Toll-like receptor 2 (TLR2), one of several pathogen receptors expressed. . .

DETD [0048] As described in detail below, the inventors have demonstrated that M. ***vaccae*** derivatives up- or down-regulate expression of genes encoding Notch receptors, Notch ligands, downstream targets of Notch signaling, and Notch-active glycosyltransferases. . . . of free-floating THP-1 cells in the absence of external stimuli. However, by ligating TLR2 on adjacent THP-1 cells, inactivated M. ***vaccae*** , DD-M. ***vaccae*** and AVAC bring THP-1 cells into very close contact with one another, thereby facilitating multiple productive interactions between Notch receptors. . . . loops; and other genes whose expression is influenced by Notch signaling (for example, Numb). Within this framework, recognition of M. ***vaccae*** derivatives by THP-1 cells is mediated by TLR2, and decision-making is mediated by both downstream products of TLR signaling (changes. . . .

DETD [0049] As used herein the term "inactivated M. ***vaccae*** " refers to M. ***vaccae*** cells that have either been killed by means of heat, as detailed below in Example 1, or by exposure to. . . . .sup.60Cobalt at a dose of 2.5 megarads, or by any other inactivation technique. As used herein, the term "modified M. ***vaccae*** " includes ***delipidated*** M. ***vaccae*** cells, ***deglycolipidated*** M. ***vaccae*** cells, M. ***vaccae*** cells that have been both ***delipidated*** and ***deglycolipidated*** (DD-M. ***vaccae***), and derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells. DD-M. ***vaccae*** may be prepared as described below in Example 1, with the preparation of derivatives of DD-M. ***vaccae*** being detailed below in Example 2. The preparation of ***delipidated*** and ***deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis) is described in Example 5, below. Derivatives of DD-M. tuberculosis and. . . . K-treated, and/or hydrofluoric acid-treated derivatives, may be prepared using the procedures disclosed herein for the preparation of derivatives of DD-M. ***vaccae*** .

DETD [0050] The derivatives of DD-M. ***vaccae*** preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of DD-M. ***vaccae*** preferably contain glucosamine in an amount greater than 3.7% of total carbohydrate, more preferably greater than 5% total carbohydrate, and most preferably greater than 7.5% total carbohydrate. Derivatives prepared by treatment of DD-M. ***vaccae*** with alkali, such as DD-M. ***vaccae*** -KOH (also known as KVAC), have a reduced number of ester bonds linking mycolic acids to the arabinogalactan of the cell wall compared to DD-M. ***vaccae*** , and are thus depleted of mycolic acids. Derivatives prepared by treatment with acid, such as DD-M. ***vaccae*** -acid (also referred to as AVAC), have a reduced number of phosphodiester bonds attaching arabinogalactan sidechains to the peptidoglycan of the. . . and are therefore depleted of arabinogalactan. In addition, such derivatives are depleted of DNA. Derivatives prepared by treatment of DD-M. ***vaccae*** with periodate, such as DD-M. ***vaccae*** -periodate (also known as IVAC), have a reduced number of cis-diol-containing sugar residues compared to DD-M. ***vaccae*** and are depleted of arabinogalactan. Derivatives prepared by treatment of DD-M. ***vaccae*** with Proteinase K (such as the derivative referred to as EVAC) are depleted of proteins and peptides. Derivatives prepared by treatment with hydrofluoric acid, such as DD-M. ***vaccae*** -KOH treated with hydrofluoric acid (referred to as HVAC), are depleted of glycosidic bonds.

DETD . . . may be effectively employed in the inventive methods include polypeptides that comprise at least a functional portion of an M. ***vaccae*** antigen, or a variant thereof. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full. . . entirely of the functional portion, or may contain

additional sequences. The additional sequences may be derived from the native M. ***vaccae*** antigen or may be heterologous.

DETD [0066] In general, M. ***vaccae*** antigens, and polynucleotides encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***vaccae*** culture filtrate. Antigens may also be produced recombinantly by inserting a DNA sequence that encodes the antigen into an expression. . .

DETD [0067] Polynucleotides encoding M. ***vaccae*** antigens may be obtained by screening an appropriate M. ***vaccae*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from amino acid sequences of isolated. . . cDNA or genomic DNA library. The library screen may then be performed using the isolated probe. DNA molecules encoding M. ***vaccae*** antigens may also be isolated by screening an appropriate M. ***vaccae*** expression library with anti-sera (e.g., rabbit or monkey) raised specifically against M. ***vaccae*** antigens.

DETD [0069] Portions and other variants of M. ***vaccae*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0074] The preferred frequency of administration and effective dosage will vary from one individual to another. For both DD-M. ***vaccae*** and derivatives of DD-M. ***vaccae*** , the amount present in a dose preferably ranges from about 10 .mu.g to about 1000 .mu.g, more preferably from about. . .

DETD Preparation of ***Delipidated*** and ***Deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***)

DETD [0077] This example illustrates the processing of different constituents of M. ***vaccae*** and their immune modulating properties.

DETD [0078] Heat-killed M. ***vaccae*** and M. ***vaccae*** Culture Filtrate

DETD [0079] M. ***vaccae*** (American Type Culture Collection Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose. . . removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10.sup.10 M. ***vaccae*** organisms per ml. The cell suspension was then autoclaved for 15 min at 120.degree. C. The culture filtrate was passaged. . .

DETD [0080] Preparation of ***Delipidated*** and ***Deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***) and Compositional Analysis

DETD [0081] To prepare ***delipidated*** M. ***vaccae*** , the autoclaved M. ***vaccae*** was pelleted by centrifugation, the pellet washed with water and collected again by centrifugation, and freeze-dried. An aliquot of this freeze-dried M. ***vaccae*** was set aside and referred to as lyophilised M. ***vaccae*** . When used in experiments it was resuspended in PBS to the desired concentration. Freeze-dried M. ***vaccae*** was treated with chloroform/methanol (2:1) for 60 min at room temperature to extract lipids, and the extraction was repeated once. The ***delipidated*** residue from the chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of M. ***vaccae*** glycolipids. The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** prepared was equivalent to 11.1% of the starting wet weight of M. ***vaccae*** used. For bioassay, the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***), was resuspended in phosphate-buffered saline by sonication, and sterilized by autoclaving.

DETD [0082] The compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae*** are presented in Table 1. Major changes are seen in the fatty acid composition and amino acid composition of DD-M.

vaccae as compared to the insoluble fraction of heat-killed M. ***vaccae*** . The data presented in Table 1 show that the insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-M. ***vaccae*** contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

TABLE 1

Compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae***

	M. ***vaccae***	DD-M. ***vaccae***
MONOSACCHARIDE COMPOSITION		
sugar alditol		
Inositol	3.2%	1.7%
Ribitol*	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%
Fatty Acid Composition		
Fatty acid		
C14:0	3.9%. . . 171	
LEU	209	340
TYR	39	75
PHE	76	132
GlcNH2	5	6
HIS	44	77
LYS	108	167
ARG	147	272

The insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and DD-M. ***vaccae*** contains 1.3% w/w of lipid.

The total amino acid content of the insoluble fraction of heat-killed M. ***vaccae*** is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-M. ***vaccae*** is 4250 nmoles/mg, or approximately 51% w/w.

DETD [0083] M. ***vaccae*** Glycolipids

DETD . . . evaporation, redissolved in water, and freeze-dried. The amount of glycolipid recovered was 1.2% of the starting wet weight of M. ***vaccae*** used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

DETD Preparation and Characterization of Additional Derivatives of M. ***vaccae***

DETD [0085] Alkaline Hydrolysis of DD-M. ***vaccae***

DETD [0087] One gram of DD-M. ***vaccae*** , prepared as described in Example 1, was suspended in 20 ml of a 0.5% solution of potassium hydroxide (KOH) in. . . ethanol and once with diethyl ether. The product was air-dried overnight. The yield was 1.01 g (101%) of KOH-treated DD-M. ***vaccae*** , subsequently referred to as DD-M. ***vaccae*** -KOH (also known as KVAC). This derivative was found to be more soluble than the other derivatives of DD-M. ***vaccae*** disclosed herein.

DETD [0088] Acid Hydrolysis of DD-M. ***vaccae***

DETD [0090] DD-M. ***vaccae*** or DD-M. ***vaccae*** -KOH (100 mg) was washed twice in 1 ml of 50 mM H.sub.2SO.sub.4 followed by resuspension and centrifugation. Other acids are. . . acid was removed by washing the residue five times with water. The freeze-dried solid residue yielded 58.2 mg acid-treated DD-M. ***vaccae*** (DD-M. ***vaccae*** -acid; also known as AVAC) or 36.7 mg acid-treated DD-M. ***vaccae*** -KOH (DD-M. ***vaccae*** -KOH-acid).

DETD [0091] Periodic Acid Cleavage of DD-M. ***vaccae***

DETD [0092] This procedure is intended to cleave cis-diol-containing sugar residues in DD-M. ***vaccae*** , such as the rhamnose residue near the attachment site of the arabinogalactan chains to the peptidoglycan backbone.

DETD [0093] DD-M. ***vaccae*** or DD-M. ***vaccae*** -KOH (100 mg) was suspended in 1 ml of a solution of 1% periodic acid in 3% acetic acid, incubated for. . . centrifugation, the solid residue was washed four times with water and freeze-dried to give a yield of 62.8 mg DD-M. ***vaccae*** -periodate (also known as IVAC) or 61.0 mg DD-M. ***vaccae*** -KOH-periodate.

DETD [0094] Resuspension of DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH

DETD [0095] DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH (11 mg each) were suspended in phosphate-buffered saline (5.5 ml). Samples were sonicated with a Virtis probe sonicator for various. . . of large particles. The absorbance of the remaining suspension at 600 nm was measured. As shown in FIG. 1, DD-M. ***vaccae*** -KOH (referred to in FIG. 1 as DDMV-KOH) was fully resuspended after one minute's sonication, and further sonication produced no further increase in the absorbance. After five minutes sonication, the resuspension of DD-M. ***vaccae*** (referred to in FIG. 1 as DDMV) was still incomplete as estimated from the absorbance of the suspension. These results indicate that DD-M. ***vaccae*** -KOH is considerably more soluble than DD-M. ***vaccae*** .

DETD [0096] Proteinase K Hydrolysis of DD-M. ***vaccae***

DETD [0098] One hundred milligrams of DD-M. ***vaccae*** , prepared as described in Example 1, was suspended in 9 ml water with sonication. Sodium dodecyl sulfate (SDS) was added. . . by centrifugation, washed with phosphate-buffered saline and water, and lyophilized. The yield was 59 mg (59%) of Proteinase K-treated DD-M. ***vaccae*** , subsequently referred to as EVAC.

DETD [0099] Hydrofluoric Acid Hydrolysis of KOH-treated DD-M. ***vaccae***

DETD [0101] One gram of DD-M. ***vaccae*** -KOH, prepared as described above, was suspended in 15 ml liquid hydrogen fluoride containing anisole as a free-radical scavenger. The mixture. . . water-insoluble fractions. The yield was 250 mg (25%) of water-soluble material, and 550 mg (55%) of water-insoluble HF-hydrolyzed KOH-treated DD-M. ***vaccae*** , subsequently referred to as HVAC.

DETD [0102] Carbohydrate Compositional Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** Derivatives

DETD [0103] The carbohydrate composition of DD-M. ***vaccae*** and DD-M. ***vaccae*** derivatives was determined using standard techniques. The results are shown in Table 2, wherein DDMV represents DD-M. ***vaccae*** ; DDMV-KOH represents DD-M. ***vaccae*** -KOH; DDMV-A represents DD-M. ***vaccae*** -acid; DDMV-I represents DD-M. ***vaccae*** -periodate; DDMV-KOH-A represents DD-M. ***vaccae*** -KOH-acid; and DDMV-KOH-I represents DD-M. ***vaccae*** -KOH-periodate.

TABLE 2

Carbohydrate Compositional Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** Derivatives

Carbohydrate	DDMV	DDMV-KOH	DDMV-A	DDMV-I	DDMV-KOH-A	DDMV-KOH-I
Galactosamine	26.6*	29.2	14.9	37.7	0.3	3.9
Glucosamine	3.7	3.6	8.7	35.6.	.	

DETD [0104] The results demonstrate that each of the DD-M. ***vaccae***

derivatives had a different carbohydrate content, as expected from the different effects of the acid, periodate or alkali treatment of the cells. In addition, DD-M. ***vaccae*** had a marked different carbohydrate composition when compared with the DD-M. ***vaccae*** derivatives. As expected, the amount of galactose in the DD-M. ***vaccae*** -acid and DD-M. ***vaccae*** -periodate derivatives was lower than in DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH. These values reflect the action of the acid and periodate in the preparation of the derivatives, cleaving the arabinogalactan sidechains. . .

DETD [0105] Nucleic Acid Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** Derivatives

DETD [0106] Analysis by gel electrophoresis of the nucleic acid content of DD-M. ***vaccae*** and the DD-M. ***vaccae*** derivatives after treatment with Proteinase K showed that DD-M. ***vaccae*** , DD-M. ***vaccae*** -periodate and DD-M. ***vaccae*** -KOH contained small amounts of DNA while no detectable nucleic acid was observed for DD-M. ***vaccae*** -acid.

DETD Effect of Immunization with DD-M. ***vaccae*** and Derivatives of DD-M. ***vaccae*** on ***Asthma*** in Mice

DETD [0107] The ability of DD-M. ***vaccae*** and derivatives of DD-M. ***vaccae*** to inhibit the development of allergic immune responses was examined in a mouse model of the ***asthma*** -like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate. . .

DETD [0109] DD-M. ***vaccae*** derivatives were prepared as described above. Groups of 10 mice were administered 200 .mu.g of PBS, DD-M. ***vaccae*** or one of the DD-M. ***vaccae*** derivatives (Q1: DD-M. ***vaccae*** ; Q2: DD-M. ***vaccae*** -KOH; Q3: DD-M. ***vaccae*** -acid; Q4: M. ***vaccae*** -periodate; Q6 and P6: DD-M. ***vaccae*** -KOH-periodate; P5: DD-M. ***vaccae*** -KOH-acid) intranasally one week before intranasal challenge with ovalbumin. As shown in FIG. 2, statistically significant reductions were observed in the. . . days after challenge with ovalbumin, compared to control mice. Furthermore, the data shows that suppression of airway eosinophilia with DD-M. ***vaccae*** -acid and DD-M. ***vaccae*** -KOH-periodate (Q3, Q6 and P6) was greater than that obtained with DD-M. ***vaccae*** (Q1). Control mice were given intranasal PBS. The data in FIG. 2 shows the mean and SEM per group of. . .

DETD [0110] Eosinophils are blood cells that are prominent in the airways in allergic ***asthma*** . The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic ***asthma*** . The data shown in FIG. 2 indicate that treatment with DD-M. ***vaccae*** or derivatives of DD-M. ***vaccae*** reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-M. ***vaccae*** or derivatives of DD-M. ***vaccae*** may therefore reduce the severity of ***asthma*** and diseases that involve similar immune abnormalities, such as allergic rhinitis, atopic dermatitis and eczema.

DETD [0111] In addition, serum samples were collected from mice immunized with either heat-killed M. ***vaccae*** or DD-M. ***vaccae*** and the level of antibodies to ovalbumin was measured by standard enzyme-linked immunoassay (EIA). As shown in Table 3 below,. . . with BCG had higher levels of ovalbumin-specific IgG1 than sera from PBS controls. In contrast, mice immunized with heat-killed M. ***vaccae*** or DD-M. ***vaccae*** had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of DD-M. ***vaccae*** on the ***asthma*** -inducing Th2 immune responses.

TABLE 3

Low Antigen-Specific IgG1 Serum Levels in Mice Immunized with Heat-killed M. ***vaccae*** or DD-M. ***vaccae***

Treatment Group	Serum IgG1 Mean	SEM
M. ***vaccae*** i.n.	185.00	8.3
M. ***vaccae*** s.c.	113.64	8.0
DD-M. ***vaccae*** i.n.	96.00	8.1
DD-M. ***vaccae*** s.c.	110.00	4.1
BCG, Pasteur	337.00	27.2
BCG, Connaught	248.00	46.1
PBS	177.14	11.4

DETD [0112] In further studies, the effects of DD-M. ***vaccae*** -acid (AVAC) on eosinophilia in the mouse model when administered either one day before challenge with OVA, at the time of. . .

DETD Effect of DD-M. ***vaccae*** Derivatives on IL-10 Production in THP-1 Cells

DETD . . . The levels of IL-10 produced by a human monocytic cell line (THP-1) cultured in the presence of derivatives of DD-M. ***vaccae*** were assessed as follows.

DETD . . . cell concentration and viability determined by staining with Trypan blue (Sigma, St Louis Mo.) and analysis under a hemocytometer. DD-M. ***vaccae*** derivatives (prepared as described above) in 50 .mu.l PBS and control stimulants were added in triplicate to wells of a. . . San Diego Calif.) according to the manufacturer's protocol. As shown in FIG. 4, the acid and periodate derivatives of DD-M. ***vaccae*** were found to stimulate significant levels of IL-10 production. The PBS control, DD-M. ***vaccae*** -KOH, DD-M. ***vaccae*** -KOH-periodate, and DD-M. ***vaccae*** -KOH-acid derivatives did not stimulate THP-1 cells to produce IL-10.

DETD Preparation and Compositional Analysis of ***Delipidated*** and ***Deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis)

DETD [0116] Cultures of Mycobacterium smegmatis (M. smegmatis, ATCC Number 27199) were grown as described in Example 1 for M. ***vaccae*** in Medium 90 with 1% added glucose. After incubation at 37.degree. C. for 5 days, the cells were harvested by. . .

DETD [0118] Preparation of ***Delipidated*** and ***Deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and ***Delipidated*** and ***Deglycolipidated*** M. smegmatis (DD-M. smegmatis) and Compositional Analysis.

DETD [0119] To prepare ***delipidated*** and ***deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis), autoclaved M. tuberculosis and M. smegmatis were pelleted by centrifugation, the. . .

DETD [0120] ***Delipidated*** and ***deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis) were prepared as described in Example 1 for the preparation of DD-M. ***vaccae*** . For bioassay, the freeze-dried DD-M. tuberculosis and DD-M. smegmatis were resuspended in phosphate-buffered saline (PBS) by sonication, and sterilized by. . .

DETD . . . in some components of the monosaccharide composition of DD-M. tuberculosis and DD-M. smegmatis compared with the monosaccharide composition of DD-M. ***vaccae*** . The data presented in Table 4 show that DD-M. tuberculosis and DD-M. smegmatis contain 1.3% and 0.0 mol % glucose, respectively, compared with 28.1 mol % for DD-M. ***vaccae*** .

DETD . . . contains 6007.7 nmoles/mg amino acids, which is approximately 72.1% w/w protein. When compared with the amino acid analysis of DD-M. ***vaccae*** , DD-M. tuberculosis and DD-M. smegmatis contain more total % protein than DD-M. ***vaccae*** (55.1%).

TABLE 4

Monosaccharide Composition of DD-M. tuberculosis and DD-M. smegmatis

Monosaccharide	M. tuberculosis wt %	M. tuberculosis mol %	M. smegmatis wt. . .

DETD Effect of Immunization with DD-M. tuberculosis and DD-M. smegmatis on ***Asthma*** in Mice

DETD . . . tuberculosis and DD-M. smegmatis to inhibit the development of allergic immune responses was examined in a mouse model of the ***asthma*** -like allergen-specific lung disease, as described above in Example 3. The results illustrate the effect of immunization with DD-M. tuberculosis and. . .

DETD . . . and 7. On days 14 and 21, mice were anesthetized and vaccinated intranasally or intradermally with 200 .mu.g of DD-M. ***vaccae*** , DD-M. tuberculosis, DD-M. smegmatis or PBS. On days 28 and 32, mice were anesthetized and challenged intranasally with 100 .mu.g. . .

DETD . . . with DD-M. tuberculosis and DD-M. smegmatis reduces the accumulation of lung eosinophils similar to the reduction following immunization with DD-M. ***vaccae*** , and that DD-M. tuberculosis and DD-M. smegmatis may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-M. tuberculosis and DD-M. smegmatis may therefore reduce the severity of ***asthma*** and diseases that involve similar immune abnormalities, such as allergic rhinitis.

DETD Effect of DD-M. ***vaccae*** on Cyctokine Production in Human Peripheral Blood Mononuclear Cells

DETD [0127] This example describes studies on the ability of DD-M. ***vaccae*** to stimulate production of IL-10, TNF-.alpha. and IFN-.gamma. in human peripheral blood mononuclear cells (PBMC).

DETD . . . blood was separated into PBMC and non-adherent cells, and the cytokine production of each fraction determined after stimulation with DD-M. ***vaccae*** as follows. Blood was diluted with an equal volume of saline and 15-20 ml was layered onto 10 ml Ficoll. . .

DETD . . . were removed, counted and resuspended at a concentration of 2.times.10.sup.6 per ml in supplemented RPMI medium. Serial dilutions of DD-M. ***vaccae*** were prepared starting at 200 .mu.g/ml and added to 100 .mu.l medium (supplemented RPMI) in a 96-well plate. PBMC and. . . was removed from each well to determine the amount of cytokine produced by the different cells after stimulation with DD-M. ***vaccae*** .

DETD [0130] DD-M. ***vaccae*** stimulated PBMC to secrete TNF-.alpha. and IL-10 (FIGS. 6 and 7A, respectively), but stimulated the non-adherent cells to produce IFN-.gamma. (FIG. 7B). These data suggest that IFN-.gamma. production in DD-M. ***vaccae*** -stimulated PBMC is repressed by the simultaneous secretion of IL-10.

DETD Effect of Intradermal Injection of Heat-Killed Mycobacterium ***vaccae*** on Psoriasis in Human Patients

DETD [0131] This example illustrates the effect of two intradermal injections of heat-killed Mycobacterium ***vaccae*** on psoriasis.

DETD [0132] M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose, 1 g/l) at. . . removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10.sup.10 M. ***vaccae*** organisms per ml. The cell suspension was then autoclaved for 15 min at 120.degree. C. and stored frozen at -20.degree. C. Prior to use the M. ***vaccae*** suspension was thawed, diluted to a concentration of 5 mg/ml in phosphate buffered saline, autoclaved for 15 min at 120.degree.. . .

DETD [0134] The 24 patients were then injected intradermally with 0.1 ml M. ***vaccae*** (equivalent to 500 .mu.g). This was followed three weeks later with a second intradermal injection with the same dose of M.

vaccae (500 .mu.g). Psoriasis was evaluated from four weeks before the first injection of heat-killed M. ***vaccae*** to twelve weeks after the first injection as follows:

DETD . . . the age, sex and clinical background of each patient.

TABLE 6

Patient Data in the Study of the Effect of M. ***vaccae*** in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-001	D. C.	49/F	30 years	28.8
PS-002	E. S.	41/F. . .		

DETD . . . at the injection site, 48 hours, 72 hours and 7 days after the first and second injections of heat-killed M. ***vaccae*** . The data shown in Table 8, below, are the PASI scores of the patients at the time of the first injection of M. ***vaccae*** (Day 0) and 3, 6, 9, 12 and 24 weeks later.

DETD [0140] It can clearly be seen that, by week 9 after the first injection of M. ***vaccae*** , 16 of 24 patients showed a significant improvement in PASI scores. Seven of 14 patients who completed 24 weeks of. . . no clinical sign of redevelopment of severe disease. These results demonstrate the effectiveness of multiple intradermal injections of inactivated M. ***vaccae*** in the treatment of psoriasis. PASI scores below 10 reflect widespread healing of lesions. Histopathology of skin biopsies indicated that. . .

DETD [0141]

TABLE 8

Clinical Status of Patients after Injection of M. ***vaccae*** (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 9	Week 12	Week 24
PS-001	28.8	14.5	10.7	2.2	0.7. . .	11.1
8.3						
PS-023	12.6	9.2	6.6	5.0	4.8	
PS-024	29.5	27.5	20.9	19.0	29.8	

*Patient PS-005 received only one dose of autoclaved M. ***vaccae*** .
**Patient PS-012 removed from trial, drug (penicillin) induced dermatitis
***Patient PS-014 was revaccinated
DNR = Did not report
Blank cells. . .

DETD Effect of Intradermal Injection of ***Delipidated*** and ***Deglycolipidated*** Mycobacterium ***vaccae*** (DD-M. ***vaccae***) on Psoriasis in Human Patients

DETD [0142] This example illustrates the effect of two intradermal injections of DD-M. ***vaccae*** on psoriasis and the lack of T cell proliferation induced in these patients after treatment with DDMV.

DETD . . . not have systemic anti-psoriasis treatment or effective topical therapy. The 17 patients were then injected intradermally with 0.1 ml DD-M. ***vaccae*** (equivalent to 100 .mu.g). This was followed three weeks later with a second intradermal injection with the same dose of DD-M. ***vaccae*** (100 .mu.g).

DETD [0144] Psoriasis was evaluated from four weeks before the first injection of M. ***vaccae*** to 48 weeks after the first injection as follows:

DETD . . . describe the age, sex and clinical background of each patient.

TABLE 9

Patient Data in the Study of the Effect

of DD-M. ***vaccae*** in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-025	A. S	25/F	2 years	12.2
PS-026	M. B	45/F. . .		

DETD . . . measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first injection of DD-M. ***vaccae*** , and 48 hours and 72 hours after the second injection.

TABLE 10

Skin Reaction Measurements in Millimeters

Time of Measurement

First. . .

DETD . . . in Table 11 are the PASI scores of the 17 patients at the time of the first injection of DD-M. ***vaccae*** (Day 0), then 3, 6, 12, 24, 36 and 48 weeks later, when available.

TABLE 11

Clinical Status of Patients after Injection of DD-M. ***vaccae*** (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 12	Week 24	Week 36	Repeat Week 48 treatment
PS-025	12.2	4.1. . .					

DETD [0151] These results show the significant improvement in PASI scores in 16 patients after injection with DD-M. ***vaccae*** . One patient dropped out of the study at 12 weeks with the diagnosis of exfoliative dermatitis/psoriasis. Patients who relapsed received a second or third injection of DD-M. ***vaccae*** at the time indicated in Table 11.

DETD . . . of these patients showed significant clinical improvement with reduction in PASI score to less than 2. Local injections of DD-M. ***vaccae*** were observed to result in clearance of skin lesions distant from the site of injection.

DETD . . . serum, penicillin (60 mg/ml), streptomycin (100 mg/ml), and glutamine (2 mM) with DDMV (12.5 and 6.25 .mu.g), or heat killed M. ***vaccae*** (6.25, 12.5, 25 or 50 .mu.g/ml) or PPD (10 or 1 .mu.g).

DETD . . . 12 shows that treatment with DDMV at 0 weeks did not enhance T cell proliferative response to DDMV nor M. ***vaccae*** 6 to 15 weeks later. Generally, treatment with DDMV also did not enhance T cell responses to PPD. Cells from. . . mitogen control, phytohemagglutinnin (PHA).

TABLE 12

Induction of T-cell proliferation in peripheral blood cells from patients treated with DDMV.

Patient No	Time after injection	PPD 10 .mu.g	1 .mu.g	M. ***vaccae*** 50 .mu.g	25 .mu.g	12.5 .mu.g	6.25 .mu.g	DDMV 12.5 .mu.g. . .	6.25 PHA

DETD Immunogenicity and Immunomodulating Properties of Recombinant Proteins Derived from M. ***vaccae*** and DD-M. ***vaccae***

DETD [0158] The polynucleotide sequences for the M. ***vaccae*** antigens GV-1/70, GV-1/83, GV-3, GV4P, GV-5, GV-5P, GV-7, GV-9, GV-13, GV-14, GV-22B, GV-23, GV-24B, GV-27, GV-27A, GV-27B, GV-29, GV-33, GV-35,. . .

DETD [0159] The immunogenicity of Mycobacterium ***vaccae*** recombinant

proteins (referred to herein as GV recombinant proteins) was tested by injecting female BALB/cByJ mice in each hind foot-pad. . .

DETD [0163] The ability of recombinant M. ***vaccae*** proteins, heat-killed M. ***vaccae*** and DD-M. ***vaccae*** to activate lymphocyte subpopulations was determined by examining upregulation of expression of CD69 (a surface protein expressed on activated cells).

DETD [0164] PBMC from normal donors (5.times.10.sup.6 cells/ml) were stimulated with 20 ug/ml of either heat-killed M. ***vaccae*** cells, DD-M. ***vaccae*** or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 24 hours. CD69 expression was determined by staining cultured cells. . .

DETD . . . Table 15 shows the percentage of .alpha..beta.T cells, .gamma..delta.T cells and NK cells expressing CD69 following stimulation with heat-killed M. ***vaccae*** , DD-M. ***vaccae*** or recombinant M. ***vaccae*** proteins. These results demonstrate that heat-killed M. ***vaccae*** , DD-M. ***vaccae*** and GV-23 stimulate the expression of CD69 in the lymphocyte subpopulations tested compared with control (non-stimulated cells), with particularly high. .

.

TABLE 15

Stimulation of CD69 Expression

	.alpha..beta.T cells	.gamma..delta.T cells	NK cells
Control	3.8	6.2	4.8
Heat-killed M. ***vaccae***	8.3	10.2	40.3
DD-M. ***vaccae***	10.1	17.5	49.9
GV-22B	5.6	3.9	8.6
GV-23	5.8	10.0	46.8
GV-27	5.5	4.4	13.3
GV-27A	5.5	4.4	13.3
GV-27B. . .			

DETD [0168] The ability of recombinant M. ***vaccae*** proteins to stimulate cytokine production in PBMC was examined as follows. PBMC from normal donors (5.times.10.sup.6 cells/ml) were stimulated with 20 ug/ml of either heat-killed M. ***vaccae*** cells, DD-M. ***vaccae*** , or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 24 hours. Culture supernatants were harvested and tested for the. . . Mass.), following the manufacturer's instructions. FIGS. 9A-D show the stimulation of IL-1.beta., TNF-.alpha., IL-12 and IFN-.gamma. production, respectively. Heat-killed M. ***vaccae*** and DD-M. ***vaccae*** were found to stimulate the production of all four cytokines examined, while recombinant GV-23 and GV-45 were found to stimulate. . .

DETD [0171] The ability of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** and recombinant M. ***vaccae*** proteins to enhance the expression of the co-stimulatory molecules CD40, CD80 and CD86 on B cells, monocytes and dendritic cells. . .

DETD . . . T cells and comprising mainly B cells, monocytes and dendritic cells were stimulated with 20 ug/ml of either heat-killed M. ***vaccae*** cells, DD-M. ***vaccae*** , or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 48 hours. Stimulated cells were harvested and analyzed for up-regulation. . . of CD40, CD80 and CD86 Expression on Dendritic Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed M. ***vaccae***	6.1	3.8	1.6
DD-M. ***vaccae***	6.6	4.2	1.6
GV-22B	4.6	1.9	1.6

	GV-23	6.0	4.5	1.8
	GV-27	5.2	1.9	1.6
	GV-27A	2.3	0.9	1.0
	GV-27B. . .			

DETD . . . of CD40, CD80 and CD86 Expression on Monocytes

	CD40	CD80	CD86
Control	0	0	0
Heat-killed M. ***vaccae***	2.3	1.8	0.7
DD-M. ***vaccae***	1.9	1.5	0.7
GV-22B	0.7	0.9	1.1
GV-23	2.3	1.5	0.7
GV-27	1.5	1.4	1.2
GV-27A	1.4	1.4	1.4
GV-27B. . .			

DETD . . . CD40, CD80 and CD86 Expression on B Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed M. ***vaccae***	1.6	1.0	1.7
DD-M. ***vaccae***	1.5	0.9	1.7
GV-22B	1.1	0.9	1.2
GV-23	1.2	1.1	1.4
GV-27	1.1	0.9	1.1
GV-27A	1.0	1.1	0.9
GV-27B. . .			

DETD . . . tested. Expression levels were most increased in dendritic cells, with the highest levels of expression being obtained with heat-killed M. ***vaccae*** , DD-M. ***vaccae*** , GV-23 and GV-45. FIGS. 12A-C show the stimulation of expression of CD40, CD80 and CD86, respectively, in dendritic cells by. . .

DETD [0178] The effect of the recombinant M. ***vaccae*** protein GV-23 on the maturation and function of dendritic cells was examined as follows.

DETD Effect of Intranasal Administration of AVAC and DD-M. ***vaccae*** on Expression of Genes Involved in Notch Signaling in Mice

DETD [0184] The ability of DD-M. ***vaccae*** and AVAC to modulate expression of genes involved in Notch signaling was assessed in 6-week-old female BALB/cByJ mice as follows.

DETD [0185] Three mice per group were immunized intranasally with 50 .mu.l PBS containing 1 mg AVAC or 1 mg DD-M. ***vaccae*** . Mice were sacrificed 24 hours later and lung samples from the mice were snap-frozen in liquid nitrogen for RNA extraction.. . .

DETD [0186] As shown in FIG. 16, real-time PCR analysis revealed that treatment of mice with AVAC and DD-M. ***vaccae*** (referred to as PVAC in FIG. 16) caused TGF.beta.1 gene expression to be significantly induced in comparison to the control. . . anti-inflammatory. HES-5 gene expression was suppressed in the AVAC treated group (.about.4 fold) and was not detectable in the DD-M. ***vaccae*** treated group. Deltex gene expression was suppressed in the presence of AVAC and DD-M. ***vaccae*** .

DETD Effect of M. ***vaccae*** , DD-M. ***vaccae*** , AVAC and M. ***vaccae*** Glycolipids on Expression of Cytokines and Genes Involved in Notch Signaling in Human Cells

DETD [0187] The ability of inactivated M. ***vaccae*** , DD-M. ***vaccae*** , AVAC and M. ***vaccae*** glycolipids to modulate expression of genes involved in Notch signaling, cytokines and Toll-like receptors (TLR) was assessed as follows using. . .

DETD . . . ml in 6-well plates. After saving an aliquot of THP-1 cells for reference purposes (t=0 hr baseline control), inactivated M. ***vaccae*** , DD-M. ***vaccae*** , AVAC or M. ***vaccae***

glycolipids was added to the cell suspension to achieve a final concentration of 100 .mu.g/ml. The cells were subsequently cultured. . .

DETD [0190] Table 20 summarizes the effects of inactivated M. ***vaccae*** , DD-M. ***vaccae*** , AVAC, and M. ***vaccae*** glycolipids on the expression of genes involved in Notch signaling in THP-1 cells.

TABLE 20

Notch signaling gene	Relative expression* M. ***vaccae***		DD-M. ***vaccae***		AVAC	Glycolipids	LPS
Notch1	1.90	1.60	3.20	1.90	2.30		
Notch2	1.40	1.10	1.40	1.20	1.40		
Notch3	5.00	--	15.1	1.90	2.30		
Notch4	0.06	0.16. . .					

DETD [0191] As shown in Table 20, M. ***vaccae*** upregulated Notch3, Delta1, Delta-like4, HES1, Deltex, HERP2, and Lunatic fringe expression; DD-M. ***vaccae*** upregulated Delta-like4, HES1, Deltex and Lunatic fringe expression; AVAC upregulated Notch1, Notch3, (Delta1), Delta-like4, HES1, Deltex, HERP2 and Lunatic fringe expression; and M. ***vaccae*** glycolipids upregulated Delta-like4, HES1, Deltex and Lunatic fringe expression. M. ***vaccae*** down-regulated Notch4, Jagged2, Manic fringe and HASH1 expression; DD-M. ***vaccae*** down-regulated Notch4 and HASH1; AVAC down-regulated Notch4, Manic fringe and HASH1 expression and M. ***vaccae*** glycolipids down-regulated Notch4, Jagged2 and HASH1 expression.

DETD [0192] A summary of the effects of inactivated M. ***vaccae*** , DD-M. ***vaccae*** , AVAC, and M. ***vaccae*** glycolipids on the expression of cytokines in THP-1 cells is presented in Table 21.

TABLE 21

Cytokine gene	Relative expression* M. ***vaccae***		DD-M. ***vaccae***		AVAC	Glycolipids	LPS
IL-1.beta.	4939	1097	2759	4011	246		
IL-6	260	125	130	11.6	27.1		
IL-8	3769	695	1722	284	267		
IL-10	391	17.6. . .					

DETD [0193] As shown in Table 21, M. ***vaccae*** upregulated IL-1.beta., IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNF.alpha. expression; DD-M. ***vaccae*** upregulated IL-1.beta., IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNF.alpha. expression; AVAC upregulated IL-1.beta., IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNF.alpha. expression; and M. ***vaccae*** glycolipids upregulated IL-1.beta., IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNF.alpha. expression. M. ***vaccae*** downregulated IL-12p35; DD-M. ***vaccae*** downregulated IL-12p35; AVAC downregulated IL-12p35; and M. ***vaccae*** glycolipids downregulated IL-12p35 expression.

DETD [0194] In further studies, the production of IL-12p40 protein in THP-1 cells in response to increasing concentrations of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** , AVAC and M. ***vaccae*** glycolipids was examined by ELISA as described above. As shown in FIG. 18, production of IL-12p40 was found to increase with increasing

concentrations of M. ***vaccae*** derivatives.

DETD [0195] The differential effect of M. ***vaccae*** derivatives on IL-12 and IL-23 gene expression in THP-1 cells was examined using real-time PCR as follows.

DETD . . . supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). THP-1 cells were cultured with 100 .mu.g/mL heat-killed M. ***vaccae*** , 100 .mu.g/mL DD-M. ***vaccae*** , 100 .mu.g/mL AVAC, with M. ***vaccae*** glycolipids, or with no M. ***vaccae*** derivative for 24 hours in cell culture medium in 6-well tissue culture plates at 1.times.10.sup.6 cells/mL in a final volume. . .

DETD . . . plotted using the ABI 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems). Expression data obtained for THP-1 cells cultured with M. ***vaccae*** derivatives were normalized to levels observed for THP-1 cells cultured in cRPMI-10 only, and the normalized values plotted as relative expression levels. As shown in FIG. 19, AVAC, DD-M. ***vaccae*** , heat-killed M. ***vaccae*** and M. ***vaccae*** glycolipids were shown to induce expression of IL-12p40 and IL-23p19 mRNA and to suppress expression of IL-12p35 mRNA.

DETD Effect of M. ***vaccae*** , DD-M. ***vaccae*** , AVAC and M. ***vaccae*** Glycolipids on Toll-Like Receptor Signaling in Human Cells

DETD . . . receptor TLR2 is known to mediate biological effects of mycobacteria and their products, particularly cell wall components, and since DD-M. ***vaccae*** and AVAC contain at least one known TLR2 ligand, namely peptidoglycan, the effect of M. ***vaccae*** derivatives on the expression of TLR genes in THP-1 cells was examined essentially as described above using primers and fluorogenic. . . specific for the TLR signaling genes CD14, TLR2, TLR7, TLR8 and MyD88. A summary of the effects of inactivated M. ***vaccae*** , DD-M. ***vaccae*** , AVAC, and M. ***vaccae*** glycolipids on TLR signaling in THP-1 cells is presented in Table 22.

TABLE 22

TLR signaling gene	Relative expression* M. ***vaccae*** Glycolipids		DD-M. ***vaccae*** LPS		AVAC
CD14	44.5	48.6	68.3	26.7	16.3
TLR2	1.9	2.0	1.0	1.7	1.7
TLR7	2.0	5.5	1.7	11.4	4.2
TLR8	42.6	77.2. . .			

DETD [0200] These results demonstrate that M. ***vaccae*** upregulated CD14 and MyD88 expression; DD-M. ***vaccae*** upregulated CD14, TLR7 and TLR8 expression; AVAC upregulated CD14, TLR8 expression; and M. ***vaccae*** glycolipids upregulated CD14, TLR7 and TLR8 expression.

DETD . . . to TLR2, TLR4 and CD14 on the production of IL-12p40, IL-10 and TNF-.alpha. in THP-1 cells in response to M. ***vaccae*** derivatives was examined as follows.

DETD . . . (Gibco BRL Life Technologies) supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). Prior to culture with M. ***vaccae*** derivatives, 50 .mu.L of THP-1 cells in cRPMI-10 were pre-treated in duplicate microplate wells with 50 .mu.L of serially diluted. . .

DETD [0203] Following pretreatment with mAbs, THP-1 cells were cultured with 5 .mu.g/mL heat-killed M. ***vaccae*** (MV), 5 .mu.g/mL DD-M. ***vaccae*** , 5 .mu.g/mL AVAC, or with no M. ***vaccae*** derivative for 24 hours in cell culture medium in 96-well round-bottom microculture plates at 1.times.10.sup.6 cells/mL in a final volume. .

.

DETD [0205] The production of IL-12p40 by THP-1 cells cultured with neutralizing antibodies and either heat-killed M. ***vaccae*** , DD-M. ***vaccae*** or AVAC is shown in FIGS. 20A-C, respectively. These figures show that M. ***vaccae*** -, AVAC- and DD-M. ***vaccae*** -induced production of IL-12p40 is inhibited by TLR2 and CD14 mAbs in a dose-dependent fashion. The production of TNF.alpha. by THP-1 cells cultured with neutralizing antibodies and either heat-killed M. ***vaccae*** , DD-M. ***vaccae*** or LPS is shown in FIGS. 21A-C, respectively. FIG. 22 shows the production of IL-10 by THP-1 cells cultured with neutralizing antibodies and heat-killed M. ***vaccae*** . These results provide evidence that M. ***vaccae*** derivatives elicit production of cytokines through Toll-like receptor signaling.

DETD Effect of M. ***vaccae*** , DD-M. ***vaccae*** , AVAC and M. ***vaccae*** Glycolipids on MRP8 Signaling in Human Cells

DETD [0206] The effect of M. ***vaccae*** derivatives on MRP8 (S100A8) signaling in THP-1 cells was determined essentially as described above using primers and fluorogenic probes for MRP8. The results are shown in Table 23.

TABLE 23

Relative expression of MRP8

M.	***vaccae***	DD-M	***vaccae***	AVAC	Glycolipids	LPS
44.5	48.6	68.3	26.7	16.3		

*Normalized relative expression of MRP8 gene mRNA in stimulus vs. medium control samples at. . .

DETD [0207] These results demonstrate that M. ***vaccae*** , DD-M. ***vaccae*** , AVAC, M. ***vaccae*** glycolipids all upregulate expression of MRP8 (S100A8). MRP-8 is a calcium-binding protein associated with psoriasis and other inflammatory skin disorders.. . .

DETD . . . to remove inhibitor or control chemicals. The THP-1 cells were then cultured with 25 .mu.g/mL AVAC, or with no M. ***vaccae*** derivative for 24 hours in cell culture medium in 96-well round-bottom microculture plates at 1.times.10.sup.6 cells/mL in a final volume. . .

11

gatgtcacgc ccggagaatg taacgttcga ccggagaacg ccgtcggcac aacgagttac 60
gtttgagcac ttcagatctc ggttaccttg gatttcaggc gggggaagca gtaaccgatc 120
caagattcga aggacccaaa caacatgaaa ttcactggaa tgaccgtgcg. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 12
LENGTH: 1518
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 12

cactcgccat gggtgttaca ataccccacc agttcctcga agtaaacgaa cagaaccgtg 60
acatccagct gagaaaatat tcacagcgac gaagcccggc cgatgcctga tggggtccgg 120
catcagtaca gcgcgctttc ctgcgcggat tctattgtcg agtccggggt. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 13
LENGTH: 1111
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 13

gtccgacagt gggacctcga gcaccacgtc acaggacagc ggccccgcca gcggcgccct 60
gcgcgtctcc aactggccgc tctatatggc cgacggtttc atcgcagcgt tccagaccgc 120
ctcgggcatc acggtcgact acaaagaaga cttcaacgac aacgagcagt. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 14
LENGTH: 1626
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 14
atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac 60
gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120
aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 15
LENGTH: 647
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 15
atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac 60
gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120
aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 16
LENGTH: 985
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 16
ggatccctac atcctgctgg tcagctccaa ggtgtcgacc gtcaaggatc tgctcccgct 60
gctggagaag gtcatccagg ccggcaagcc gctgctgatc atcgccgagg acgtcgaggg 120
cgaggccctg tccacgctgg tggtcaacaa gatccgcggc accttcaagt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 17
LENGTH: 743
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 17
ggatccgcgg caccggctgg tgacgaccaa gtacaacccg gcccgcacct ggacggccga 60
gaactccgtc ggcatcggcg gcgcgtacct gtgcatctac gggatggagg gccccggcgg 120
ctatcagttc gtcggccgca ccacccaggt gtggagtcgt taccgccaca. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 18
LENGTH: 1164
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 18
ggtggcgcgc atcgagaagc gcccgccccg gttcacgggc gcctgatcat ggtgcgggcg 60
gcgctgcgct acggcttcgg gacggcctca ctgctggccg gcgggttcgt gctgcgcgcc 120
ctgcagggca cgcctgccgc cctcggcgcg actccgggcg aggtcgcgcc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 1012
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 19
atgaaggcaa atcattcggg atgctacaaa tccgccggcc cgatatggtc gcatccatcg 60
ccgctttgtt cgcccgcact ggcaccatct catgcaggtc tggacaatga gctgagcctg 120
ggcatccacg gccagggccc ggaacgactg accattcagc agtgggacac. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 898
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 20
gagcaaccgt tccggctcgg cgactggatc accgtcccca ccgcggcggg ccggccgtcc 60
gcccacggcc gcgtggtgga agtcaactgg cgtgcaacac atatcgacac cggcggcaac 120
ctgctggtaa tgcccaacgc cgaactcgcc ggcgcgtcgt tcaccaatta. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 21
LENGTH: 2013
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 21
ggctatcagt ccggacggtc ctcgctgcgc gcatcggtgt tcgaccgcct caccgacatc 60
cgcgagtcgc agtcgcgcgg gttggagaat cagttcgcgg acctgaagaa ctcgatggtg 120
atttactcgc gcggcagcac tgccacggag gcgatcggcg cgttcagcga. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 522
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 22
acctacgagt tcgagaacaa ggtcacgggc ggccgcatcc cgcgcgagta catcccgtcg 60
gtggatgccg gcgcgcagga cgccatgcag tacggcgtgc tggccggcta cccgctggtt 120
aacgtcaagc tgacgctgct cgacggtgcc taccacgaag tcgactcgtc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 23
LENGTH: 570
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 23
agacagacag tgatcgacga aaccctcttc catgccgagg agaagatgga gaaggccgtc 60
tcggtggcac ccgacgacct ggcgtcgatt cgtaccggcc gcgcgaaccc cggcatgttc 120
aaccggatca acatcgacta ctacggcgcc tccaccccga tcacgcagct. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 24
LENGTH: 1071
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 24
cgtggggaag gattgcactc tatgagcgaa atcgcccgtc cctggcgggt tctggcaggt 60
ggcatcggtg cctgcgccgc gggtatcgcc ggggtgctga gcatcgcggt caccacggcg 120
tcggcccagc cgggcctccc gcagcccccg ctgcccgccc ctgccacagt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 25
LENGTH: 1364
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 25
cgacctccac ccgggcgtga ggccaaccac taggctggtc accagtagtc gacggcacac 60
ttcaccgaaa aaatgaggac agaggagaca cccgtgacga tccgtgttgg tgtgaacggc 120
ttcggccgta tcggacgcaa cttcttccgc gcgctggacg cgcagaaggc. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 26
LENGTH: 858
TYPE: DNA
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 26
gaaatcccgc gtctgaaacc ctcttttcgc ggcgcccctc aggacggtaa gggggccaag 60
cggattgaaa aatgttcgct gaatgagcct gaaattgcgc gtggctcttg gaaatcagca 120
gcgatgggtt taccgtgtcc actagtcggt ccaaagagga ccactggttt. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 231
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 27
Asp Thr Val Leu Met Pro Pro Ala Asn Asn Arg Arg Ser Ser Thr Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28

LENGTH: 228
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 28
Met Met Thr Thr Arg Arg Lys Ser Ala Ala Val Ala Gly Ile Ala Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 326
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 29
Met Arg Leu Leu Asp Arg Ile Arg Gly Pro Trp Ala Arg Arg Phe Gly
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 161
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 30
Ser Gly Trp Asp Ile Asn Thr Ala Ala Phe Glu Trp Tyr Val Asp Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 334
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 31
Met Lys Phe Thr Glu Lys Trp Arg Gly Ser Ala Lys Ala Ala Met His
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 161
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 32
Asn Gly Trp Asp Ile Asn Thr Pro Ala Phe Glu Trp Phe Tyr Glu Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 33
LENGTH: 142
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 33
Met Arg Thr Ala Thr Thr Lys Leu Gly Ala Ala Leu Gly Ala Ala Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 34
LENGTH: 285
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 34
Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 35
LENGTH: 159
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 35
Met Thr Ala Gly Ala Ala Ala Ala Ala Thr Leu Gly Ala Ala Ala Val
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 36
LENGTH: 166
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 36
Met Pro Val Arg Arg Ala Arg Ser Ala Leu Ala Ser Val Thr Phe Val
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 37
LENGTH: 136
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 37
Met Lys Phe Thr Gly Met Thr Val Arg Ala Ser Arg Arg Ala Leu Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 38
LENGTH: 376
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 38
Val Ile Glu Ile Asp His Val Thr Lys Arg Phe Gly Asp Tyr Leu Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 39
LENGTH: 348
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 39
Ser Asp Ser Gly Thr Ser Ser Thr Thr Ser Gln Asp Ser Gly Pro Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 541
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 40
Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 215
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 41
Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 327
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 42
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 43
LENGTH: 243
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 43
Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr
1 5. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 370
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 44
Met Val Arg Ala Ala Leu Arg Tyr Gly Phe Gly Thr Ala Ser Leu Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 45
LENGTH: 336
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 45
Met Lys Ala Asn His Ser Gly Cys Tyr Lys Ser Ala Gly Pro Ile Trp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 297
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
FEATURE:
NAME/KEY: VARIANT
LOCATION: (1)...(297)
OTHER INFORMATION: Xaa = Any Amino Acid
SEQUENCE: 46
Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 47
LENGTH: 670
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 47
Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser Val Phe Asp Arg
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 48
LENGTH: 173
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 48
Thr Tyr Glu Phe Glu Asn Lys Val Thr Gly Gly Arg Ile Pro Arg Glu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 49
LENGTH: 187
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
FEATURE:
NAME/KEY: VARIANT
LOCATION: (1)...(187)
OTHER INFORMATION: Xaa = Any Amino Acid
SEQUENCE: 49
Val Ile Asp Glu Thr Leu Phe His Ala Glu Glu. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 50
LENGTH: 331
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 50
Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Gly Gly Ile Gly
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 51
LENGTH: 340
TYPE: PRT
ORGANISM: Mycobacterium ***vaccae***
SEQUENCE: 51
Val Thr Ile Arg Val Gly Val Asn Gly Phe Gly Arg Ile Gly Arg Asn
1 5. . .

CLM What is claimed is:

. . . antigen presenting cells with a composition comprising at least one component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . administering to the subject a composition comprising at least one component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . administering to the subject a composition comprising at least one component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and

treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . administering to the subject a composition comprising at least one component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . contacting the cells with a composition comprising at least one component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . population of cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of: (a) ***delipidated*** and ***deglycolipidated*** M. smegmatis cells; and (b) ***delipidated*** and ***deglycolipidated*** M. tuberculosis cells.

. . . cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae***

cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

. . . cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of: (a) inactivated M. ***vaccae*** cells; (b) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells; (c) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; (d) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; (e) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid; (f) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis; (g) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid; (h) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with Proteinase K; and (i) ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by hydrofluoric acid hydrolysis.

L7 ANSWER 6 OF 10 USPATFULL on STN
AN 2003:153650 USPATFULL
TI DNA encoding SNORF33 receptor
IN Borowsky, Beth E., Montclair, NJ, UNITED STATES
Ogozalek, Kristine L., Rochelle Park, NJ, UNITED STATES
Jones, Kenneth A., Bergenfield, NJ, UNITED STATES
PA Synpatic Pharmaceutical Corporation (U.S. corporation)
PI US 2003105318 A1 20030605
AI US 2002-267217 A1 20021007 (10)
RLI Continuation of Ser. No. US 1999-413433, filed on 6 Oct 1999, ABANDONED
Continuation-in-part of Ser. No. US 1999-322257, filed on 28 May 1999, ABANDONED
DT Utility

FS APPLICATION
LREP John P. White, Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY, 10036
CLMN Number of Claims: 160
ECL Exemplary Claim: 1
DRWN 23 Drawing Page(s)
LN.CNT 4917
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides isolated nucleic acids encoding mammalian SNORF33 receptors, purified mammalian SNORF33 receptors, vectors comprising nucleic acid encoding mammalian SNORF33 receptors, cells comprising such vectors, antibodies directed to mammalian SNORF33 receptors, nucleic acid probes useful for detecting nucleic acid encoding mammalian SNORF33 receptors, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding mammalian SNORF33 receptors, transgenic, nonhuman animals which express DNA encoding normal or mutant mammalian SNORF33 receptors, methods of isolating mammalian SNORF33 receptors, methods of treating an abnormality that is linked to the activity of the mammalian SNORF33 receptors, as well as methods of determining binding of compounds to mammalian SNORF33 receptors, methods of identifying agonists and antagonists of SNORF33 receptors, and agonists and antagonists so identified.
SUMM . . . sites for [.sup.3H]p-tyramine have been reported in rat brain, which may reflect specific TYR receptor sites (Ungar et al., 1977; ***Vaccaria*** , 1986; ***Vaccaria*** , 1988). Further studies are needed before a clear definition of specifc p-tyramine binding site is available. There are no reports. . .
SUMM . . . reacting substance of anaphylaxis that has prostaglandin E-like activity (Bakhle and Smith, 1977). Therefore, T may have a function in ***asthma*** .
DETD . . . AIDS, pain, psychotic and neurological disorders, including anxiety, depression, schizophrenia, dementia, mental retardation, memory loss, epilepsy, neuromotor disorders, respiratory disorders, ***asthma*** , eating/body weight disorders including obesity, bulimia, diabetes, anorexia, nausea, hypertension, hypotension, vascular and cardiovascular disorders, ischemia, stroke, cancers, ulcers, urinary. . .
DETD . . . disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, ***asthma*** , a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a. . .
DETD . . . disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, ***asthma*** , a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a. . .
DETD . . . 5 mm dithiothreitol, rinsed in DEPC-treated water, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed twice in 2.times.SSC, ***delipidated*** with chloroform then dehydrated through a series of graded alcohols. All reagents were purchased from Sigma (St. Louis, Mo.).
DETD . . . kidney.

Region	qRT-PCR % of max	Potential applications
heart	1.19	Cardiovascular indications
kidney	100	Hypertension, electrolyte balance
liver	9.71	Diabetes
lung	2.45	Respiratory disorders, ***asthma***

pancreas	1.34	Diabetes, endocrine disorders
pituitary	2.04	Endocrine/neuroendocrine regulation
placenta	0.44	Gestational abnormalities
small intestine	44.22	Gastrointestinal disorders
spleen	1.98	Immune disorders
stomach	88.02	Gastrointestinal disorders

striated. . .
DETD . . . disorders

heart	0.06	cardiovascular indications
hippocampus	not detected	cognition/memory
hypothalamus	0.10	appetite/obesity, neuroendocrine regulation
kidney	0.05	electrolyte balance, hypertension
liver	0.32	diabetes
lung	0.21	respiratory disorders, ***asthma***
medulla	0.71	analgesia, modulation of autonomic function, sensory transmission and modulation
nucleus accumbens	not detected	regulation of dopaminergic function, drug addiction, neuropsychiatric. . .

DETD [0539] ***Vaccari*** , A., "High affinity binding of [.sup.3H]-tyramine in the central nervous system", Br. J. Pharmacol. 89: 15-25 (1986).
DETD [0540] ***Vaccari*** , A., "High affinity binding of p-tyramine: A process in

L7 ANSWER 7 OF 10 USPATFULL on STN
AN 2003:78030 USPATFULL
TI Individualization of therapy with hyperlipidemia agents
IN Leyland-Jones, Brian, Miami, FL, UNITED STATES
PA McGill University, Montreal, CANADA (U.S. corporation)
PI US 2003053950 A1 20030320
AI US 2002-125690 A1 20020417 (10)
PRAI US 2001-284210P 20010418 (60)
DT Utility
FS APPLICATION
LREP HAMILTON, BROOK, SMITH & REYNOLDS, P.C., 530 VIRGINIA ROAD, P.O. BOX 9133, CONCORD, MA, 01742-9133
CLMN Number of Claims: 113
ECL Exemplary Claim: 1
DRWN 24 Drawing Page(s)
LN.CNT 5288
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the individualization of therapy on the basis of a phenotypic profile of an individual. More specifically, the present invention relates to the use of metabolic phenotyping for the individualization of treatment with hyperlipidemia agents.
SUMM . . . the family included pyruvate aminotransferase, branched-chain amino acid aminotransferase, tyrosine aminotransferase, aromatic aminotransferase, alanine: glyoxylate aminotransferase (AGT), and kynurenine aminotransferase (***Vacca*** , R. A. et al. (1997) J. Biol. Chem. 272: 21932-21937).
SUMM . . . PDE4s have been studied extensively as possible targets for novel anti-inflammatory agents, with special emphasis placed on the discovery of ***asthma*** treatments. PDE4 inhibitors are currently undergoing clinical trials as treatments for ***asthma*** , chronic obstructive pulmonary disease, and atopic eczema. All four known isozymes of PDE4 are susceptible to the inhibitor rolipram, a. . .
SUMM [0101] Theophylline is a nonspecific PDE inhibitor used in the treatment

of bronchial ***asthma*** and other respiratory diseases. Theophylline is believed to act on airway smooth muscle function and in an anti-inflammatory or immunomodulatory. . .

DETD . . . VLDL is the first step in endogenous lipid metabolism, a reduction in VLDL production results in decreased formation of its ***delipidated*** products, such as IDL and LDL particles. The increase in HDL cholesterol level results mostly from the concomitant reduction in. . .

DETD . . . of the need for phenotyping in drug dosing is the case of theophylline. Theophylline is used in the treatment of ***asthma*** . However, theophylline toxicity continues to be a common clinical problem, and involves life-threatening cardiovascular and neurological toxicity. Theophylline is cleared. . .

L7 ANSWER 8 OF 10 USPATFULL on STN

AN 2003:30886 USPATFULL

TI Compositions isolated from skin cells and methods for their use

IN Watson, James D., Auckland, NEW ZEALAND
Strachan, Lorna, Auckland, NEW ZEALAND
Sleeman, Matthew, Weston Colville, UNITED KINGDOM
Onrust, Rene, Mercer Is, WA, UNITED STATES
Murison, James G., Auckland, NEW ZEALAND
Kumble, Krishanand D., Los Altos, CA, UNITED STATES

PA Genesis Research and Development Corporation Limited, Auckland, NEW ZEALAND (non-U.S. corporation)

PI US 2003022835 A1 20030130

AI US 2002-152661 A1 20020520 (10)

RLI Continuation-in-part of Ser. No. US 2001-866050, filed on 24 May 2001, PENDING Continuation-in-part of Ser. No. US 1999-312283, filed on 14 May 1999, PENDING Continuation-in-part of Ser. No. US 1998-188930, filed on 9 Nov 1998, GRANTED, Pat. No. US 6150502 Continuation-in-part of Ser. No. US 1998-69726, filed on 29 Apr 1998, ABANDONED

PRAI WO 1999-NZ51 19990429
US 2000-206650P 20000524 (60)
US 2000-221232P 20000725 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 29

ECL Exemplary Claim: 1

DRWN 14 Drawing Page(s)

LN.CNT 3053

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Isolated polynucleotides encoding polypeptides expressed in mammalian skin cells are provided, together with expression vectors and host cells comprising such isolated polynucleotides. Methods for the use of such polynucleotides and polypeptides are also provided.

DETD . . . neuronal cell
migration and cancers, agents for the healing of cancers, neuro-degenerative diseases, wound healing, inflammatory autoimmune diseases like psoriasis, ***asthma*** , Crohus disease
and as agents for the prevention of HTV-1 of leukocytes

264 341 Nucleotide-sugar transporter family member.

365 389 Transforming growth. . .

DETD [0180] Skin biopsies taken from psoriasis patients before and after treatment with ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (U.S. Pat. Nos. 5,985,287 and 6,328,978) were analyzed for TR1 expression. All of the differentiating keratinocytes expressed TR1, whereas the. . .

DETD . . . neuronal cells migration and cancers; as agents for the treatment of cancers, neuro-degenerative diseases, inflammatory autoimmune diseases such as psoriasis, ***asthma*** and Crohn's

disease; for use in wound healing; and as agents for the prevention of HIV-1 binding and infection of. . .

L7 ANSWER 9 OF 10 USPATFULL on STN
AN 2002:343542 USPATFULL
TI Methods and compounds for the treatment of immunologically - mediated diseases of the respiratory system using mycobacterium ***vaccae***
IN Watson, James D., Auckland, NEW ZEALAND
Tan, Paul L.J., Auckland, NEW ZEALAND
PI US 2002197265 A1 20021226
AI US 2002-51643 A1 20020118 (10)
RLI Continuation of Ser. No. US 1998-156181, filed on 17 Sep 1998, PENDING Continuation-in-part of Ser. No. US 1997-996624, filed on 23 Dec 1997, ABANDONED
DT Utility
FS APPLICATION
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CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 10 Drawing Page(s)
LN.CNT 6136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment by immunotherapy of lung immune disorders, including infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such components.
TI Methods and compounds for the treatment of immunologically - mediated diseases of the respiratory system using mycobacterium ***vaccae***
AB . . . and treatment by immunotherapy of lung immune disorders, including infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising administering a composition having antigenic and/or adjuvant properties. Compositions which may be usefully employed in the inventive methods include inactivated M. ***vaccae*** cells, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, M. ***vaccae*** culture filtrate and compounds present in or derived therefrom, together with combinations of such components.
SUMM . . . system which result from immune disorders. In particular, the invention is related to the use of compositions comprising inactivated Mycobacterium ***vaccae*** (M. ***vaccae***), and/or compounds prepared from M. ***vaccae*** for the treatment and prevention of respiratory and/or lung disorders including mycobacterial infections, such as Mycobacterium tuberculosis and Mycobacterium avium, and for the treatment of disorders, such as sarcoidosis, ***asthma*** and lung cancers.
SUMM . . . well-known mycobacterium that has been used for immunotherapy for tuberculosis and also leprosy, by subcutaneous or intradermal injection, is Mycobacterium ***vaccae*** (M. ***vaccae***), which is non-pathogenic in humans. However, there is less information on the efficacy of M. ***vaccae*** compared with BCG, and it has not been used widely to vaccinate the general public. M. bovis BCG and M. ***vaccae*** are believed to contain antigenic compounds that are recognized by the immune system of individuals exposed to infection with M.. . .
SUMM [0007] Several patents and other publications disclose treatment of

various conditions by administering mycobacteria, including M. ***vaccae*** , or certain mycobacterial fractions. U.S. Pat. No. 4,716,038 discloses diagnosis of, vaccination against and treatment of autoimmune diseases of various types, including arthritic diseases, by administering mycobacteria, including M. ***vaccae*** . U.S. Pat. No. 4,724,144 discloses an immunotherapeutic agent comprising antigenic material derived from M. ***vaccae*** for treatment of mycobacterial diseases, especially tuberculosis and leprosy, and as an adjuvant to chemotherapy. International Patent Publication WO 91/01751 discloses the use of antigenic and/or immunoregulatory material from M. ***vaccae*** as an immunoprophylactic to delay and/or prevent the onset of AIDS. International Patent Publication WO 94/06466 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for therapy of HIV infection, with or without AIDS and with or without associated tuberculosis.

SUMM [0008] U.S. Pat. No. 5,599,545 discloses the use of mycobacteria, especially whole, inactivated M. ***vaccae*** , as an adjuvant for administration with antigens which are not endogenous to M. ***vaccae*** . This publication theorises that the beneficial effect as an adjuvant may be due to heat shock protein 65 (hsp 65). International Patent Publication WO 92/08484 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for the treatment of uveitis. International Patent Publication WO 93/16727 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for the treatment of mental diseases associated with an autoimmune reaction initiated by an infection. International Patent Publication WO 95/26742 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for delaying or preventing the growth or spread of tumours. International Patent Publication WO 91/02542 discloses the use of autoclaved M. ***vaccae*** in the treatment of chronic inflammatory disorders in which a patient demonstrates an abnormally high release of IL-6 and/or TNF. . .

SUMM [0009] M. ***vaccae*** is apparently unique among known mycobacterial species in that heat-killed preparations retain vaccine and immunotherapeutic properties. For example, M. tuberculosis. . .

SUMM . . . N-glycolymuramic acid in approximately equimolar amounts) as described in U.S. Pat. Nos. 3,956,481 and 4,036,953. These compounds differ from the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***) of the present invention in the following aspects of their composition:

SUMM [0011] 1. They are water-soluble agents, whereas DD-M. ***vaccae*** is insoluble in aqueous solutions.

SUMM . . . unit, either extracted from bacteria by various solvents, or digested from the cell wall by an enzyme. In contrast, DD-M. ***vaccae*** contains highly polymerised cell wall.

SUMM . . . of the cell wall peptidoglycan structure, namely alanine, glutamic acid, diaminopimelic acid, N-acetyl glucosamine, and N-glycolylmuramic acid. In contrast, DD-M. ***vaccae*** contains 50% w/w protein, comprising a number of distinct protein species.

SUMM [0017] ***Asthma*** is a common disease, with a high prevalence in the developed world. ***Asthma*** is characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli, the primary physiological disturbance being reversible airflow limitation, which may be spontaneous or drug-related, and the pathological hallmark being inflammation of the airways. Clinically, ***asthma*** can be subdivided into extrinsic and intrinsic variants.

SUMM [0018] Extrinsic ***asthma*** has an identifiable precipitant, and can be thought of as being atopic, occupational and drug-induced. Atopic ***asthma*** is associated with the enhancement of a Th2-type of immune response with the production of specific immunoglobulin E (IgE), positive. . . be divided further into seasonal and perennial forms according to the seasonal timing of symptoms. The airflow obstruction in

extrinsic ***asthma*** is due to nonspecific bronchial hyperesponsiveness caused by inflammation of the airways. This inflammation is mediated by chemicals released by. . . and lymphocytes. The actions of these mediators result in vascular permeability, mucus secretion and bronchial smooth muscle constriction. In atopic ***asthma*** , the immune response producing airway inflammation is brought about by the Th2 class of T cells which secrete IL-4, IL-5 and IL-10. It has been shown that lymphocytes from the lungs of atopic ***asthmatics*** produce IL-4 and IL-5 when activated. Both IL-4 and IL-5 are cytokines of the Th2 class and are required for the production of IgE and involvement of eosinophils in ***asthma*** . Occupational ***asthma*** may be related to the development of IgE to a protein hapten, such as acid anhydrides in plastic workers and plicatic acid in some western red cedar-induced ***asthma*** , or to non-IgE related mechanisms, such as that seen in toluene diisocyanate-induced ***asthma*** . Drug-induced ***asthma*** can be seen after the administration of aspirin or other non-steroidal anti-inflammatory drugs, most often in a certain subset of patients who may display other features such as nasal polyposis and sinusitis. Intrinsic or cryptogenic ***asthma*** is reported to develop after upper respiratory tract infections, but can arise de novo in middle-aged or older people, in whom it is more difficult to treat than extrinsic ***asthma*** .

SUMM [0019] ***Asthma*** is ideally prevented by the avoidance of triggering allergens but this is not always possible nor are triggering allergens always easily identified. The medical therapy of ***asthma*** is based on the use of corticosteroids and bronchodilator drugs to reduce inflammation and reverse airway obstruction. In chronic ***asthma*** , treatment with corticosteroids leads to unacceptable adverse side effects.

SUMM [0020] Another disorder with a similar immune abnormality to ***asthma*** is allergic rhinitis. Allergic rhinitis is a common disorder and is estimated to affect at least 10% of the population.. . .

SUMM . . . specific against the allergen. The inflammatory response occurs in the nasal mucosa rather than further down the airways as in ***asthma*** . Like ***asthma*** , local eosinophilia in the affected tissues is a major feature of allergic rhinitis. As a result of this inflammation, patients. . . and interfere with a person's ability to work. Current treatment involves the use of antihistamines, nasal decongestants and, as for ***asthma*** , sodium cromoglycate and corticosteroids.

SUMM . . . immunotherapy of immune disorders of the respiratory system, including infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers. The inventive methods comprise administering a composition having antigenic and/or adjuvant properties. In one aspect. . .

SUMM . . . which may be usefully employed in the inventive methods comprise a component selected from the group consisting of inactivated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, and combinations thereof.

SUMM . . . comprise administering one or more doses of a composition including a component selected from the group consisting of inactivated M. ***vaccae*** cells, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, and components that are present in or derived from either M. ***vaccae*** cells or M. ***vaccae*** culture filtrate. Specific examples of components present in or derived from either M. ***vaccae*** cells or M. ***vaccae*** culture filtrate include isolated polypeptides that comprise a sequence selected from the group consisting of SEQ ID NO: 1-4, 9-16,. . .

SUMM . . . compositions which may be usefully employed in such methods comprise a component selected from the group consisting of inactivated

M. ***vaccae*** cells, M. ***vaccae*** culture filtrate, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, and constituents and combinations thereof.

SUMM . . . employed in the present invention may additionally include a non-specific immune response enhancer, or adjuvant. Such adjuvants may include M. ***vaccae*** culture filtrate, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, or an isolated polypeptide comprising a sequence provided in SEQ ID NO: 89, 117, 160, 162 or 201, or. . .

SUMM . . . disorder is characterized by the presence of eosinophilia in the tissues of the respiratory system. Examples of such diseases include ***asthma*** and allergic rhinitis. In a related aspect, the present invention provides methods for the reduction of eosinophilia, in a patient,. . .

DRWD [0034] FIG. 1 compares the stimulation of IL-12 production in macrophages by different concentrations of heat-killed M. ***vaccae*** , lyophilized M. ***vaccae*** , ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** and M. ***vaccae*** glycolipids.

DRWD . . . FIG. 2 compares the stimulation of interferon-gamma production in spleen cells from SCID mice by different concentrations of heat-killed M. ***vaccae*** , lyophilized M. ***vaccae*** , ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** and M. ***vaccae*** glycolipids.

DRWD [0036] FIGS. 3A(i)-(iv) illustrate the non-specific immune amplifying effects of 10 .mu.g, 100 .mu.g and 1 mg autoclaved M. ***vaccae*** and 75 .mu.g unfractionated culture filtrates of M. ***vaccae*** , respectively. FIGS. 3B(i) and (ii) illustrate the non-specific immune amplifying effects of autoclaved M. ***vaccae*** , and ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** , respectively. FIG. 3C(i) illustrates the non-specific immune amplifying effects of whole autoclaved M. ***vaccae*** . FIG. 3C(ii) illustrates the non-specific immune amplifying effects of soluble M. ***vaccae*** proteins extracted with SDS from ***delipidated*** and ***deglycolipidated*** M ***vaccae*** . FIG. 3C(iii) illustrates that the adjuvant effect of the preparation of FIG. 3C(ii) is destroyed by treatment with the proteolytic enzyme pronase. FIG. 3D illustrates the non-specific immune amplifying effects of heat-killed M. ***vaccae*** (FIG. 3D(i)), whereas heat-killed preparations of M. tuberculosis (FIG. 3D(ii)), M. bovis BCG (FIG. 3D(iii)), M. phlei (FIG. 3D(iv)) and. . .

DRWD . . . and B show the percentage of eosinophils in mice immunized intranasally with either 10 or 1000 .mu.g of heat-killed M. ***vaccae*** or 200-100 .mu.g of DD-M. ***vaccae*** , respectively, 4 weeks prior to challenge with ovalbumin, as compared to control mice. FIGS. 4C and D show the percentage of eosinophils in mice immunized intranasally with either 100 .mu.g of heat-killed M. ***vaccae*** or 200 .mu.g of DD-M. ***vaccae*** , respectively, as late as one week prior to challenge with ovalbumin. FIG. 4E shows the percentage of eosinophils in mice. . . (s.c.) with either BCG of the Pasteur strain (BCG-P), BCG of the Connought strain (BCG-C), 1 mg of heat-killed M. ***vaccae*** , or 200 .mu.g of DD-M. ***vaccae*** prior to challenge with ovalbumin.

DETD . . . T cell immune responses and protective immunity against M. tuberculosis in rodents and non-human primates following immunization with heat-killed M. ***vaccae*** and various M. ***vaccae*** derivatives through the lung. The inventors have additionally demonstrated that both heat-killed M. ***vaccae*** and M. ***vaccae*** derivatives are able to inhibit the development of an allergic immune response in the lungs when administered either intranasally or subcutaneously in a rodent model of ***asthma*** .

DETD . . . central to a reversal of disease state in many disorders, including disorders of the respiratory system such as tuberculosis,

sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers.

DETD [0043] Inactivated M. ***vaccae*** and compounds derived from M. ***vaccae*** have both antigenic and adjuvant properties. The methods of the present invention employ compounds from M. ***vaccae*** and/or its culture filtrates that have T cell enhancing immune activities. Mixtures of such compounds are particularly useful in redirecting. . . contain many cross-reacting antigens, it is not known whether they contain adjuvant compounds in common. As shown below, inactivated M. ***vaccae*** cells and a modified (***delipidated*** and ***deglycolipidated***) form of M. ***vaccae*** have been found to have adjuvant properties which are not shared by a number of other mycobacterial species. Furthermore, it has been found that M. ***vaccae*** produces compounds in its own culture filtrate which amplify a Th1-type immune response to M. ***vaccae*** antigens also found in culture filtrate, as well as to antigens from other sources.

DETD [0044] The present invention provides methods for the immunotherapy of respiratory and/or lung disorders, including tuberculosis, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers, in a patient to enhance Th1-type immune responses. In one embodiment, the compositions are delivered. . . Compositions which may be usefully employed in the inventive methods comprise at least one of the following components: inactivated M. ***vaccae*** cells; M. ***vaccae*** culture filtrate; ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells (DD-M. ***vaccae***); and compounds present in or derived from M. ***vaccae*** and/or its culture filtrate. As illustrated below, administration of such compositions, results in specific T cell immune responses and enhanced protection against M. tuberculosis infection. Administration of such compositions is also effective in the treatment of ***asthma*** . While the precise mode of action of these compositions in the treatment of diseases such as ***asthma*** is unknown, they are believed to suppress an ***asthma*** -inducing Th2 immune response.

DETD [0045] Inactivated M. ***vaccae*** are M. ***vaccae*** that have either been killed by means of heat, as detailed below, or subjected to radiation, such as .sup.60cobalt at. . . of 2.5 megarads. As detailed in Example 3, the inventors have shown that removal of the glycolipid constituents from M. ***vaccae*** results in the removal of molecular components that stimulate interferon-gamma production in natural killer (NK) cells, thereby significantly reducing the. . .

DETD [0049] As used herein the term "inactivated M. ***vaccae*** " refers to M. ***vaccae*** cells that have either been killed by means of heat, as detailed below in Examples 1 and 2, or subjected to radiation, such as .sup.60Cobalt at a dose of 2.5 megarads. As used herein, the term "modified M. ***vaccae*** " includes ***delipidated*** M. ***vaccae*** cells, ***deglycolipidated*** M. ***vaccae*** cells and M. ***vaccae*** cells that have been both ***delipidated*** and ***deglycolipidated*** .

DETD [0050] ***Delipidated*** and ***deglycolipidated*** M. ***vaccae*** may be prepared as described below in Example 1. As detailed below, the inventors have shown that removal of the glycolipid constituents from M. ***vaccae*** results in the removal of molecular components that stimulate interferon-gamma production in natural killer (NK) cells, thereby significantly reducing the. . .

DETD [0051] Compounds present in or derived from M. ***vaccae*** cells and/or from M. ***vaccae*** culture filtrate that may be usefully employed in the inventive methods include M. ***vaccae*** polypeptides, or variants thereof. Such polypeptides possess antigenic and/or adjuvant properties. In specific embodiments, such polypeptides comprise a sequence selected. . .

DETD . . . entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***vaccae*** antigen or may be heterologous, and such sequences may

(but need not) be immunogenic. As detailed below, polypeptides of the present invention may be isolated from M. ***vaccae*** cells or culture filtrate, or may be prepared by synthetic or recombinant means.

DETD [0055] A M. ***vaccae*** adjuvant is a compound found in M. ***vaccae*** cells or M. ***vaccae*** culture filtrates which non-specifically stimulates immune responses. Adjuvants enhance the immune response to immunogenic antigens and the process of memory formation. In the case of M. ***vaccae*** proteins, these memory responses favor Th1-type immunity. Adjuvants are also capable of stimulating interleukin-12 production or interferon-.gamma. production in biological. . . and macrophages, where the cells are derived from healthy individuals. Adjuvants may or may not stimulate cell proliferation. Such M. ***vaccae*** adjuvants include, for example, polypeptides comprising a sequence recited in SEQ ID NO: 89, 117, 160, 162 or 201.

DETD [0078] Portions and other variants of M. ***vaccae*** polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0080] In general, M. ***vaccae*** polypeptides, and DNA sequences encoding such polypeptides, may be prepared using any of a variety of procedures. For example, soluble polypeptides may be isolated from M. ***vaccae*** culture filtrate as described below. Polypeptides may also be produced recombinantly by inserting a DNA sequence that encodes the polypeptide. . .

DETD [0081] DNA sequences encoding M. ***vaccae*** polypeptides may be obtained by screening an appropriate M. ***vaccae*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD [0082] DNA molecules encoding M. ***vaccae*** polypeptides may also be isolated by screening an appropriate M. ***vaccae*** cDNA or genomic DNA expression library with anti-sera (e.g., rabbit or monkey) raised specifically against M. ***vaccae*** polypeptides, as detailed below.

DETD [0089] For use in the inventive methods, the inactivated M. ***vaccae*** cell, M. ***vaccae*** culture filtrate, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, or compounds present in or derived from M. ***vaccae*** and/or its culture filtrate are generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or. . . and a physiologically acceptable carrier. Vaccines may comprise one or more components selected from the group consisting of inactivated M. ***vaccae*** cells, M. ***vaccae*** culture filtrate, ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, and compounds present in or derived from M. ***vaccae*** and/or its culture filtrate, together with a non-specific immune response amplifier. Such pharmaceutical compositions and vaccines may also contain other. . .

DETD . . . in an aerosol form or by means of a nebulizer device similar to those currently employed in the treatment of ***asthma*** . In other embodiments, the pharmaceutical composition or vaccine is in a form suitable for administration by injection (intracutaneous, intramuscular, intravenous. . .

DETD . . . the patient, but will typically range from about 0.1 mL to about 5 mL. In the case of inactivated M. ***vaccae*** cells, the amount present in a dose preferably ranges from about 10 to about 1000 mg, and is more preferably about 500 mg. For DD-M. ***vaccae*** , the amount present in a dose preferably ranges from about 10 .mu.g to about 1000 .mu.g, more preferably from about 50 .mu.g to about 200 .mu.g. For both inactivated M. ***vaccae*** and DD-M. ***vaccae*** , the number of doses may range from 1 to about 10 administered over a period of up to 12 months.

DETD Effect of Intradermal and Intra-lung Routes of Immunization with M.

vaccae on Tuberculosis in Cynomolgous Monkeys

DETD [0095] This example illustrates the effect of immunization with heat-killed M. ***vaccae*** or M. ***vaccae*** culture filtrate through intradermal and intralung routes in cynomolgous monkeys prior to challenge with live M. tuberculosis.

DETD [0096] M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5g/l; glucose, 1 g/l) at 37.degree.. . . removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10.sup.10 M. ***vaccae*** organisms per ml. The cell suspension was then autoclaved for 15 min at 120.degree. C. The culture filtrate was passaged. . .

DETD . . . cynomolgous monkeys were used, with each group containing 2 monkeys. Two groups of monkeys were immunized with whole heat-killed M. ***vaccae*** either intradermally or intralung; two groups of monkeys were immunized with M. ***vaccae*** culture filtrate either intradermally or intralung; and a control group received no immunizations. All immunogens were dissolved in phosphate buffered. . . post-immunization these measurements were repeated. At day 34, all monkeys received a second immunization using the same amount of M. ***vaccae*** and route of immunization as the initial immunization. On day 62, body weight, temperature, ESR and LPA to PPD were. . . Route of

Number	Monkey	Immunogen	Immunization
1	S3101-E	0	--
(Controls)	3144-B	0	--
2	4080-B	500 .mu.g	intradermal
(Immunized	3586-B	500 .mu.g	intradermal
with heat-killed M. ***vaccae***)			
3	3534-C	500 .mu.g	intralung
(Immunized	3160-A	500 .mu.g	intralung
with heat-killed M. ***vaccae***)			
4	3564-B	100 .mu.g	intradermal
(Immunized	3815-B	100 .mu.g	intradermal
with culture filtrate)			
5	4425-A	100 .mu.g	intralung
(Immunized	2779-D	100 .mu.g	intralung
with culture filtrate)			

DETD [0100] Table 2B--The two monkeys immunized twice with 500 .mu.g M. ***vaccae*** intradermally showed no sign of lung disease 84 days post-infection with M. tuberculosis. The LPA responses to PPD indicated there. . .

DETD [0101] Table 2C--The two monkeys immunized twice with 500 .mu.g M. ***vaccae*** intralung showed almost identical results to those animals of Table 2B. There was no sign of lung disease 84 days. . .

DETD [0102] Immunization twice with 500 .mu.g of whole M. ***vaccae*** has consistently shown protective effects agsint subsequent infection with live M. tuberculosis. The data presented in Tables 2D and 2E show the effects of immunization with 100 .mu.g of M. ***vaccae*** culture filtrate. Monkeys immunized intradermally showed signs of developing disease 84 days post-infection, while in those immunized intralung, one animal. . .

DETD [0103]

TABLE 2B

MONKEYS IMMUNIZED WITH WHOLE HEAT-KILLED M. ***VACCAE*** (500 .mu.g) INTRADERMAL

ID#	Days	Wt. Kgs	Temp.	ESR mm/hr	LPA PPD 10 .mu.g	LPA PPD 1 .mu.g	X-Ray Remarks

4080-B. . .
DETD [0104]
TABLE 2C

MONKEYS IMMUNIZED WITH WHOLE HEAT-KILLED
M. ***VACCAE*** (500 .mu.g) INTRALUNG

ID#	Days	Wt. Kgs	Temp.	ESR mm/hr	LPA PPD 10 .mu.g	LPA PPD 1 .mu.g	X-Ray Remarks

3534-C. . .
DETD Effect of Immunization with M. ***vaccae*** on ***Asthma*** in Mice
DETD [0107] This example illustrates that both heat-killed M. ***vaccae*** and DD-M. ***vaccae*** , when administered to mice via the intranasal route, are able to inhibit the development of an allergic immune response in the lungs. This was demonstrated in a mouse model of the ***asthma*** -like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate. . .
DETD . . . As shown in FIGS. 4A and B, groups of seven mice administered either 10 or 1000 .mu.g of heat-killed M. ***vaccae*** (FIG. 4A), or 10, 100 or 200 .mu.g of DD-M. ***vaccae*** (FIG. 4B) intranasally 4 weeks before intranasal challenge with ovalbumin, had reduced percentages of eosinophils in the BAL cells collected. . .
DETD [0110] FIGS. 4C and D show that mice given either 1000 .mu.g of heat-killed M. ***vaccae*** (FIG. 4C) or 200 .mu.g of DD-M. ***vaccae*** (FIG. 4D) intranasally as late as one week before challenge with ovalbumin had reduced percentages of eosinophils compared to control. . .
DETD [0111] As shown in FIG. 4E, immunization with either 1 mg of heat-killed M. ***vaccae*** or 200 .mu.g of DD-M. ***vaccae*** , given either intranasally (i.n.) or subcutaneously (s.c.), reduced lung eosinophilia following challenge with ovalbumin when compared to control animals given. . .
DETD [0112] Eosinophils are blood cells that are prominent in the airways in allergic ***asthma*** . The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic ***asthma*** . The data shown in FIGS. 4A-E indicate that treatment with heat-killed M. ***vaccae*** or DD-M. ***vaccae*** reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of heat-killed M. ***vaccae*** or DD-M. ***vaccae*** may therefore reduce the severity of ***asthma*** and diseases that involve similar immune abnormalities, such as allergic rhinitis.
DETD . . . with BCG had higher levels of ovalbumin specific IgG1 than sera from PBS controls. In contrast, mice immunized with M. ***vaccae*** or DD-M. ***vaccae*** had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of heat-killed M. ***vaccae*** and DD-M. ***vaccae*** on the ***asthma*** -inducing Th2 immune responses.
TABLE 3

LOW ANTIGEN-SPECIFIC IgG1 SERUM LEVELS IN MICE
IMMUNIZED WITH HEAT-KILLED M. ***VACCAE*** OR DD-M. ***VACCAE***

Treatment Group	Serum IgG1 Mean	SEM
M. ***vaccae*** i.n.	185.00	8.3
M. ***vaccae*** s.c.	113.64	8.0
DD-M. ***vaccae*** i.n.	96.00	8.1
DD-M. ***vaccae*** s.c.	110.00	4.1
BCG, Pasteur	337.00	27.2
BCG, Connaught	248.00	46.1
PBS	177.14	11.4

Note:
Ovalbumin-specific IgG1 was detected using anti-mouse. . .

DETD Preparation and Immune Modulating Properties of ***Delipidated*** and ***Deglycolipidated*** (DD-) M. ***vaccae***

DETD [0114] This example illustrates the processing of different constituents of M. ***vaccae*** and their immune modulating properties.

DETD [0115] Heat-killed M. ***vaccae*** and M. ***vaccae*** culture filtrate

DETD [0116] M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose 1 g/l) at. . . removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10.sup.10 M. ***vaccae*** organisms per ml. The cell suspension was then autoclaved for 15 min at 120.degree. C. The culture filtrate was passaged. . .

DETD [0117] ***Delipidated*** and ***Deglycolipidated*** (DD-) M. ***vaccae*** and Compositional Analysis

DETD [0118] To prepare ***delipidated*** M. ***vaccae*** , the autoclaved M. ***vaccae*** was pelleted by centrifugation, the pellet washed with water and collected again by centrifugation and then freeze-dried. Freeze-dried M. ***vaccae*** was treated with chloroform/methanol (2:1) for 60 mins at room temperature to extract lipids, and the extraction was repeated once. The ***delipidated*** residue from chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of M. ***vaccae*** glycolipids (see below). The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** prepared was equivalent to 11.1% of the starting wet weight of M. ***vaccae*** used. For bioassay, the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** , referred to as DD-M. ***vaccae*** , was resuspended in phosphate-buffered saline by sonication, and sterilized by autoclaving.

DETD [0119] The compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae*** are presented in Table 4. Major changes are seen in the fatty acid composition and amino acid composition of DD-M. ***vaccae*** as compared to the insoluble fraction of heat-killed M. ***vaccae*** . The data presented in Table 4 shows that the insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-M. ***vaccae*** contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

TABLE 4

Compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae***

	M. ***vaccae***	DD-M. ***vaccae***
Monosaccharide composition		

sugar alditol		
Inositol	3.2%	1.7%
Ribitol*	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%
Fatty acid composition		
Fatty acid		
C14:0	3.9%. . .	

DETD [0120] The insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and DD-M. ***vaccae*** contains 1.3% w/w of lipid.

Amino Acid Composition nmoles/mg	M. ***vaccae***	DD-M. ***vaccae***
ASP	231	361
THR	170	266
SER	131	199
GLU	319	505
PRO	216	262
GLY	263	404
ALA	416. . .	

DETD [0121] The total amino acid content of the insoluble fraction of heat-killed M. ***vaccae*** is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-M. ***vaccae*** is 4250 nmoles/mg, or approximately 51% w/w.

DETD [0122] Comparison of composition of DD-M. ***vaccae*** with ***delipidated*** and ***deglycolipidated*** forms of M. tuberculosis and M. smegmatis

DETD [0123] ***Delipidated*** and ***deglycolipidated*** M. tuberculosis and M. smegmatis were prepared using the procedure described above for ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** . As indicated in Table 5, the profiles of the percentage composition of amino acids in DD-M. ***vaccae*** , DD-M. tuberculosis and DD-M. smegmatis showed no significant differences. However, the total amount of protein varied--the two batches of DD-M. ***vaccae*** contained 34% and 55% protein, whereas DD-M. tuberculosis and DD-M. smegmatis contained 79% and 72% protein, respectively.

TABLE 5

Amino Acid Composition of ***Delipidated*** and ***Deglycolipidated*** Mycobacteria

Amino Acid	DD-M. ***vaccae*** Batch 1	DD-M. ***vaccae*** Batch 2	M. smegmatis	DD-M. tuberculosis
Asp	9.5	9.5	9.3	9.1
Thr	6.0	5.9	5.0	5.3
Ser	5.3	5.3. . .		

DETD [0124] Analysis of the monosaccharide composition showed significant differences between DD-M. ***vaccae*** , and DD-M. tuberculosis and DD-M. smegmatis. The monosaccharide composition of two batches of DD-M. ***vaccae*** was the same and differed from that of DD-M. tuberculosis and M. smegmatis. Specifically, DD-M. ***vaccae*** was found to contain free glucose while both DD-M. tuberculosis and M. smegmatis contain glycerol, as shown in Table 6.

TABLE 6

Alditol
Acetate wt % mol %

DD-M. ***vaccae***
Batch 1
Inositol 0.0 0.0
Arabinose 54.7 59.1
Mannose 1.7 1.5
Glucose 31.1 28.1
Galactose 12.5 11.3
100.0 100.0
Batch 2
Inositol 0.0 0.0
Arabinose 51.0 55.5
Mannose 2.0. . .

DETD [0125] M. ***vaccae*** glycolipids

DETD . . . evaporation, redissolved in water and freeze-dried. The amount of glycolipid recovered was 1.2% of the starting wet weight of M. ***vaccae*** used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

DETD [0128] Whole heat-killed M. ***vaccae*** and DD-M. ***vaccae*** were shown to have different cytokine stimulation properties. The stimulation of a Th1 immune response is enhanced by the production of interleukin-12 (IL-12) from macrophages. The ability of different M. ***vaccae*** preparations to stimulate IL-12 production was demonstrated as follows.

DETD . . . in cell culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of whole heat-killed M. ***vaccae*** , heat-killed M. ***vaccae*** which was lyophilised and reconstituted for use in phospate-buffered saline, DD-M. ***vaccae*** , or M. ***vaccae*** glycolipids were added. After three days at 37.degree. C., the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in FIG. 1, all the M. ***vaccae*** preparations stimulated the production of IL-12 from macrophages.

DETD [0130] By contrast, these same M. ***vaccae*** preparations were examined for the ability to stimulate interferon-gamma production from Natural Killer (NK) cells. Spleen cells were prepared from. . . contain 75-80% NK cells. The spleen cells were incubated at 37.degree. C. in culture with different concentrations of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** , and M. ***vaccae*** glycolipids. The data shown in FIG. 2 demonstrated that, while heat-killed M. ***vaccae*** and M. ***vaccae*** glycolipids stimulate production of interferon-gamma, DD-M. ***vaccae*** stimulated relatively less interferon gamma. The combined data from FIGS. 1 and 2 indicate that compared with M. ***vaccae*** , DD-M. ***vaccae*** was a better stimulator of IL-12 than interferon gamma.

DETD [0131] These findings demonstrate that removal of the lipid glycolipid constituents from M. ***vaccae*** results in the removal of molecular components that stimulate interferon-gamma from NK cells, thereby effectively eliminating an important cell source of a cytokine that has numerous harmful side-effects. DD-M. ***vaccae*** thus retains Th1 immune enhancing capacity by stimulating IL-12 production, but has lost the non-specific effects that may come through. . .

DETD . . . GVc-7 + +
GV-22B + ND
GV-27 + +
GV-27A + +
GV-27B + +
GV-42 + ND
DD. M. ***vaccae*** + +

ND = not done

DETD [0133] Proteins in DD-M. ***vaccae*** as non-specific immune amplifiers

DETD The Non-specific Immune Amplifying Properties of Heat-killed M. ***vaccae*** , M. ***vaccae*** Culture filtrate and DD-M. ***vaccae***

DETD [0135] This example illustrates the non-specific immune amplifying or `adjuvant` properties of whole heat-killed M. ***vaccae*** , DD-M. ***vaccae*** and M. ***vaccae*** culture filtrate.

DETD [0136] M. ***vaccae*** bacteria was cultured, pelleted and autoclaved as described in Example 1. Culture filtrates of live M. ***vaccae*** refer to the supernatant from 24 h cultures of M. ***vaccae*** in 7H9 medium with glucose. DD-M. ***vaccae*** was prepared as described in Example 3.

DETD [0137] Killed M. ***vaccae*** , DD-M. ***vaccae*** and M. ***vaccae*** culture filtrate were tested for adjuvant activity in the generation of cytotoxic T cell immune response to ovalbumin, a structurally. . . C57BL/6 mice were immunized by the intraperitoneal injection of 100 .mu.g of ovalbumin with the following test adjuvants: heat-killed M. ***vaccae*** ; DD-M. ***vaccae*** ; DD-M. ***vaccae*** with proteins extracted with SDS; the SDS protein extract treated with Pronase (an enzyme which degrades protein); and either heat-killed M. ***vaccae*** , heat-killed M. bovis BCG, M. phlei, M. smegmatis or M. ***vaccae*** culture filtrate. After 10 days, spleen cells were stimulated in vitro for a further 6 days with E.G7 cells which. . .

DETD [0139] The diagrams that make up FIG. 3 show the effect of various M. ***vaccae*** derived adjuvant preparations on the generation of cytotoxic T cells to ovalbumin in C57BL/6 mice. As shown in FIG. 3A,. . . cells were generated in mice immunized with (i) 10 .mu.g, (ii) 100 .mu.g or (iii) 1 mg of autoclaved M. ***vaccae*** or (iv) 75 .mu.g of M. ***vaccae*** culture filtrate. FIG. 3B shows that cytotoxic cells were generated in mice immunized with (i) 1 mg whole autoclaved M. ***vaccae*** or (ii) 100 .mu.g DD-M. ***vaccae*** . As shown in FIG. 3C(i), cytotoxic cells were generated in mice immunized with 1 mg heat-killed M. ***vaccae*** ; FIG. 3C(ii) shows the active material in M. ***vaccae*** soluble proteins extracted with SDS from DD-M. ***vaccae*** . FIG. 3C(iii) shows that active material in the adjuvant preparation of FIG. 3C(ii) was destroyed by treatment with the proteolytic. . . comparison, 100 .mu.g of the SDS-extracted proteins had significantly stronger immune-enhancing ability (FIG. 3C(ii)) than did 1 mg heat-killed M. ***vaccae*** (FIG. 3C(i)). Mice immunized with 1 mg heat-killed M. ***vaccae*** (FIG. 3D(i)) generated cytotoxic cells to ovalbumin, but mice immunized separately with 1 mg heat-killed M. tuberculosis (FIG. 3D(ii)), 1. . .

DETD [0140] The significance of these findings is that heat-killed M. ***vaccae*** and DD-M. ***vaccae*** have adjuvant properties not seen in other mycobacteria. Further, ***delipidation*** and ***deglycolipidation*** of M. ***vaccae*** removes an NK cell-stimulating activity but does not result in a loss of T cell-stimulating activity.

DETD . . . revealed homology to the heat shock protein 65 (GroEL) gene from M. tuberculosis, indicating that this protein is an M. ***vaccae*** member of the GroEL family.

DETD [0142] An expression library of M. ***vaccae*** genomic DNA in BamH1-lambda ZAP-Express (Stratagene) was screened using sera from cynomolgous monkeys immunized with M. tuberculosis secreted proteins prepared. . . Sanger sequencing with fluorescent primers on Perkin Elmer/Applied Biosystems Division automatic sequencer. The determined nucleotide sequence of the partial M. ***vaccae*** GroEL-homologue clone GV-27 is provided in SEQ ID NO:77 and the predicted amino acid sequence in SEQ ID NO:78. This. . .

DETD [0143] A partial sequence of the 65 kDa heat shock protein of M.

vaccae has been published by Kapur et al. (Arch. Pathol. Lab. Med. 119:131-138, 1995). However, this sequence did not overlap with. . .

DETD . . . NO: 162. The sequence of GV-27A shows 95.8% identity to the published M. tuberculosis GroEL sequence and contains the M. ***vaccae*** sequence of Kapur et al. discussed above. The sequence of GV-27B is about 92.2% identical to the published M. tuberculosis. . .

DETD Purification and Characterization of Polypeptides from M. ***vaccae*** Culture Filtrate

DETD [0146] This example illustrates the preparation of M. ***vaccae*** soluble proteins from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD [0147] M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 at 37.degree. C. The cells were harvested by centrifugation, and transferred. . .

DETD [0157] Using the procedures described above, six soluble M. ***vaccae*** antigens, designated GVc-1, GVc-2, GVc-7, GVc-13, GVc-20 and GVc-22, were isolated. Determined N-terminal and internal sequences for GVc-1 are shown. . .

DETD . . . GVc-22 was cloned. When sub-cloned into the expression vector pET16, no protein expression was obtained. Subsequent screening of the M. ***vaccae*** BamHI genomic DNA library with the incomplete gene fragment led to the isolation of the complete gene encoding GVc-22. To. . .

DETD . . . NO: 1) and the M. tuberculosis MPT70 gene sequence. Using these primers, a 310 bp fragment was amplified from M. ***vaccae*** genomic DNA and cloned into EcoRV-digested vector pBluescript II SK.sup.+ (Stratagene). The sequence of the cloned insert is provided in SEQ ID NO: 62. The insert of this clone was used to screen a M. ***vaccae*** genomic DNA library constructed in lambda ZAP-Express (Stratgene, La Jolla, Calif.). The clone isolated contained an open reading frame with. . .

DETD . . . SK.sup.+ and sequenced (SEQ ID NO: 150) following standard procedures (Maniatis). The cloned insert was used to screen a M. ***vaccae*** genomic DNA library constructed in lambda ZAP-Express. The clone isolated had homology to M. tuberculosis antigen MPT70 and M. bovis. . .

DETD . . . and their T cells were shown to proliferate in response to PPD. Donor PBMCs and crude soluble proteins from M. ***vaccae*** culture filtrate were cultured in medium comprising RPMI 1640 supplemented with 10% (v/v) autologous serum, penicillin (60 mg/ml), streptomycin (100. . .

DETD Purification and Characterisation of Polypeptides from M. ***vaccae*** Culture Filtrate by 2-Dimensional Polyacrylamide Gel Electrophoresis

DETD [0170] M. ***vaccae*** soluble proteins were isolated from culture filtrate using 2-dimensional polyacrylamide gel electrophoresis as described below. Unless otherwise noted, all percentages. . .

DETD [0171] M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 at 37.degree. C. M. tuberculosis strain H37Rv (ATCC number 27294) was. . .

DETD . . . and applied to second dimension gels (16% polyacrylamide). FIGS. 4A and 4B are the 2-D gel patterns observed with M. ***vaccae*** culture filtrate and M. tuberculosis H37Rv culture filtrate, respectively. Polypeptides from the second dimension separation were transferred to PVDF membranes. . .

DETD DNA Cloning Strategy for the M. ***Vaccae*** Antigen 85 Series

DETD . . . mycobacterial species, and between mycobacterial species. These oligonucleotides were used under reduced stringency conditions to amplify target sequences from M. ***vaccae*** genomic DNA. An appropriately-sized 485 bp band was identified, purified, and cloned pBluescript II SK.sup.+0 (Stratagene, La Jolla, Calif.). Twenty-four. . . antigen 85C gene sequences, with the remainder being more similar to antigen 85B sequences. In addition, these two putative M. ***vaccae***

antigen 85 genomic sequences were 80% homologous to one another. Because of this high similarity, the antigen 85C PCR fragment was chosen to screen M. ***vaccae*** genomic libraries at low stringency for all three antigen 85 genes.

DETD [0177] An M. ***vaccae*** genomic library was created in lambda Zap-Express (Stratagene, La Jolla, Calif.) by cloning BamHI partially-digested M. ***vaccae*** genomic DNA into similarly-digested vector, with 3.4.times.10.sup.5 independent plaque-forming units resulting. For screening purposes, twenty-seven thousand plaques from this non-amplified. . . to M. bovis and M. tuberculosis antigen 85A genes, each containing either the 5' or 3' ends of the M. ***vaccae*** gene (this gene was cleaved during library construction as it contains an internal BamHI site). The remaining clones were found. . . subclones were constructed and sequenced. Overlapping sequences were aligned using the DNA Strider software. The determined DNA sequences for M. ***vaccae*** antigens 85A, 85B and 85C are shown in SEQ ID NO: 40-42, respectively, with the predicted amino acid sequences being. . .

DETD [0178] The M. ***vaccae*** antigens GVs-3 and GVs-5 were expressed and purified as follows. Amplification primers were designed from the insert sequences of GVs-3. . . EcoRI and BamHI restriction sites were added to the primers for GVs-5 for cloning convenience. Following amplification from genomic M. ***vaccae*** DNA, fragments were cloned into the appropriate site of pProEX HT prokaryotic expression vector (Gibco BRL, Life Technologies, Gaithersburg, Md.). . .

DETD [0179] Expression of a fragment of the M. ***vaccae*** antigen GVs-4 (antigen 85B homolog) was performed as follows. The primers AD58 and AD59, described above, were used to amplify a 485 bp fragment from M. ***vaccae*** genomic DNA. This fragment was gel-purified using standard techniques and cloned into EcoRV-digested pBluescript. The base sequences of inserts from. . .

DETD DNA Cloning Strategy for M. ***vaccae*** Antigens

DETD [0182] An 84 bp probe for the M. ***vaccae*** antigen GVc-7 was amplified using degenerate oligonucleotides designed to the determined amino acid sequence of GVc-7 (SEQ ID NO: 5-8). This probe was used to screen a M. ***vaccae*** genomic DNA library as described in Example 4. The determined nucleotide sequence for GVc-7 is shown in SEQ ID NO:. . .

DETD . . . putative leader sequence. A XhoI restriction site was added to the primers for cloning convenience. Following amplification from genomic M. ***vaccae*** DNA, fragments were cloned into the XhoI-site of pProEX HT prokaryotic expression vector (Gibco BRL) and submitted for sequencing to. . .

DETD . . . MPG15) was designed to the GVs-8 peptide sequence shown in SEQ ID NO: 26 and used to screen a M. ***vaccae*** genomic DNA library using standard protocols. A genomic clone containing genes encoding four different antigens was isolated. The determined DNA. . .

DETD [0187] The gene encoding GV-33 was amplified from M. ***vaccae*** genomic DNA with primers based on the determined nucleotide sequence. This DNA fragment was cloned into EcoRv-digested pBluescript II SK.sup.+. . .

DETD DNA Cloning Strategy for the M. ***Vaccae*** Antigens GV-23, GV-24, GV-25, GV-26, GV-38A and GV-38B

DETD [0189] M. ***vaccae*** (ATCC Number 15483) was grown in sterile Medium 90 at 37.degree. C. for 4 days and harvested by centrifugation. Cells. . .

DETD [0190] Total M. tuberculosis and M. ***vaccae*** RNA was depleted of 16S and 23S ribosomal RNA (rRNA) by hybridization of the total RNA fraction to oligonucleotides AD10. . .

DETD [0191] The M. tuberculosis and M. ***vaccae*** cDNA was used as template for single-sided-specific PCR (3S-PCR). For this protocol, a degenerate oligonucleotide AD1 (SEQ ID NO:85) was. . . primer AD1 as 5'-primer and AD7 as 3'-primer, products were separated on a

urea/polyacrylamide gel. DNA bands unique to M. ***vaccae*** were excised and re-amplified using primers AD1 and AD7. After gel purification, bands were cloned into pGEM-T (Promega) and the. . .

DETD [0193] A M. ***vaccae*** genomic DNA library constructed in BamHI-digested lambda ZAP Express (Stratagene) was probed with the radiolabelled 238 bp band 12B21 following. . . phagemid containing a 4.5 kb insert was identified by Southern blotting and hybridisation. The nucleotide sequence of the full-length M. ***vaccae*** homologue of pota (ATP-binding protein) was identified by subcloning of the 4.5 kb fragment and base sequencing. The gene consisted. . . of 320 bp containing putative -10 and -35 promoter elements. The nucleotide and predicted amino acid sequences of the M. ***vaccae*** pota homologue are provided in SEQ ID NO: 88 and 89, respectively.

DETD [0194] The nucleotide sequence of the M. ***vaccae*** pota gene was used to design primers EV24 and EV25 (SEQ ID NO: 90 and 91) for expression cloning. The. . .

DETD . . . in SEQ ID NO:92. To identify the gene, the radiolabelled insert of this subclone was used to probe an M. ***vaccae*** genomic DNA library constructed in the Sal1-site of lambda Zap-Express (Stratagene) following standard protocols. A clone was identified of which 1342 bp showed homology with the potd gene of E. coli. The potd homologue of M. ***vaccae*** was identified by sub-cloning and base sequencing. The determined nucleotide and predicted amino acid sequences are shown in SEQ ID. . .

DETD [0196] For expression cloning, primers EV26 and EV27 (SEQ ID NO:95-96) were designed from the determined M. ***vaccae*** potd homologue. The amplified fragment was cloned into pProEX HT Prokaryotic expression system (Gibco BRL). Expression in an appropriate E.. . .

DETD [0199] Base sequence adjacent to the M. ***vaccae*** potd gene-homologue was found to show homology to the potb gene of the spermidine/putrescine ABC transporter complex of E.coli, which is one of two transmembrane proteins in the ABC transporter complex. The M. ***vaccae*** potb homologue (referred to as GV-25) was identified through further subcloning and base sequencing. The determined nucleotide and predicted amino. . .

DETD . . . to PotC, the second transmembrane protein of E.coli, and suggests that a second transmembrane protein is absent in the M. ***vaccae*** homologue of the ABC transporter. An open reading frame with homology to M. tuberculosis acetyl-CoA acetyl transferase, however, was identified. . .

DETD . . . above for the isolation of GV-23, the 3S-PCR band 12B28 (SEQ ID NO: 119) was used to screen the M. ***vaccae*** genomic library constructed in the BamHI-site of lambda ZAP-Express (Stratagene). The clone isolated from the library contained a novel open. . .

DETD Purification and Characterisation of Polypeptides from M. ***vaccae*** Culture Filtrate by Preparative Isoelectric Focusing and Preparative Polyacrylamide Gel Electrophoresis

DETD [0204] M. ***vaccae*** soluble proteins were isolated from culture filtrate using preparative isoelectric focusing and preparative polyacrylamide gel electrophoresis as described below. Unless. . .

DETD [0205] M. ***vaccae*** (ATCC Number 15483) was cultured in 250 l sterile Medium 90 which had been fractionated by ultrafiltration to remove all. . .

DETD . . . Murine polyclonal antisera were prepared against GV-40 and GV-44 following standard procedures. These antisera were used to screen a M. ***vaccae*** genomic DNA library consisting of randomly sheared DNA fragments. Clones encoding GV-40 and GV-44 were identified and sequenced. The determined. . .

DETD DNA Cloning Strategy for the DD-M. ***vaccae*** Antigen GV-45

DETD [0213] Proteins were extracted from DD-M. ***vaccae*** (500 mg; prepared as described above) by suspension in 10 ml 2% SDS/PBS and heating to 50.degree. C. for 2. . .

DETD . . . Jolla, Calif.) and sequenced (SEQ ID NO:190) following standard

procedures (Maniatis). The cloned insert was used to screen a M. ***vaccae*** genomic DNA library constructed in the BamHI-site of lambda ZAP-Express (Stratagene). The isolated clone showed homology to a 35 kDa. . .

CLM What is claimed is:
1. A method for the treatment of ***asthma*** in a patient, comprising administering to the patient a composition comprising ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, wherein the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells comprise less than 10% by weight of lipids.

5. A method for the treatment of ***asthma*** in a patient, comprising administering to the patient a composition comprising ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, wherein the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells comprise more than 33% by weight of amino acids.

L7 ANSWER 10 OF 10 USPATFULL on STN
AN 2002:39664 USPATFULL
TI Methods and compounds for the treatment of immunologically-mediated diseases using mycobacterium ***vaccae***
IN Watson, James D., Auckland, NEW ZEALAND
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PI US 6350457 B1 20020226
AI US 1999-449013 19991124 (9)
PRAI US 1999-137112P 19990602 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P
LREP Sleath, Janet, Speckman, Ann W.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1305
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for the prevention and treatment of disorders, including disorders of the respiratory system, such as infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising administering a composition comprising derivatives of ***delipidated*** and ***deglycolipidated*** M ***vaccae*** cells.
TI Methods and compounds for the treatment of immunologically-mediated diseases using mycobacterium ***vaccae***
AB . . . disorders, including disorders of the respiratory system, such as infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers are provided, such methods comprising administering a composition comprising derivatives of ***delipidated*** and ***deglycolipidated*** M ***vaccae*** cells.
SUMM . . . of immunologically-mediated disorders. In certain embodiments, the invention is related to the use of compositions comprising components prepared from Micobacterium ***vaccae*** , Mycobacterium tuberculosis and Mycobacterium smegmatis for the treatment of immunologically-mediated disorders of the respiratory system, such as sarcoidosis, ***asthma*** and lung cancers, for treatment of allergic disorders such as atopic dermatitis, for treatment of diseases that benefit from the. . .

SUMM A less well-known mycobacterium that has been used for immunotherapy for tuberculosis, and also leprosy, is Microbacterium ***vaccae*** (M. ***vaccae***), which is non-pathogenic in humans. However, there is less information on the efficacy of M. ***vaccae*** compared with BCG, and it has not been used widely to vaccinate the general public. M. bovis BCG and M. ***vaccae*** are believed to contain antigenic compounds that are recognized by the immune system of individuals exposed to infection with M. . .

SUMM Several patents and other publications disclose treatment of various conditions by administering mycobacteria, including M. ***vaccae*** , or certain mycobacterial fractions. U.S. Pat. No. 4,716,038 discloses diagnosis of, vaccination against and treatment of autoimmune diseases of various types, including arthritic diseases, by administering mycobacteria, including M. ***vaccae*** . U.S. Pat. No. 4,724,144 discloses an immunotherapeutic agent comprising antigenic material derived from M. ***vaccae*** for treatment of mycobacterial diseases, especially tuberculosis and leprosy, and as an adjuvant to chemotherapy. International Patent Publication WO 91/01751 discloses the use of antigenic and/or immunoregulatory material from M. ***vaccae*** as an immunoprophylactic to delay and/or prevent the onset of AIDS. International Patent Publication WO 94/06466 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for therapy of HIV infection, with or without AIDS and with or without associated tuberculosis.

SUMM U.S. Pat. No. 5,599,545 discloses the use of mycobacteria, especially whole, inactivated M. ***vaccae*** , as an adjuvant for administration with antigens that are not endogenous to M. ***vaccae*** . This publication theorizes that the beneficial effect as an adjuvant may be due to heat shock protein 65 (hsp65). International Patent Publication WO 92/08484 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for the treatment of uveitis. International Patent Publication WO 93/16727 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for the treatment of mental diseases associated with an autoimmune reaction initiated by an infection. International Patent Publication WO 95/26742 discloses the use of antigenic and/or immunoregulatory material derived from M. ***vaccae*** for delaying or preventing the growth or spread of tumors. International Patent Publication WO 91/02542 discloses the use of autoclaved M. ***vaccae*** in the treatment of chronic inflammatory disorders in which a patient demonstrates an abnormally high release of IL-6 and/or TNF. . .

SUMM M. ***vaccae*** is apparently unique among known mycobacterial species in that heat-killed preparations retain vaccine and immunotherapeutic properties. For example, M. bovis. . .

SUMM . . . N-glycolymuramic acid in approximately equimolar amounts) as described in U.S. Pat. Nos. 3,956,481 and 4,036,953. These compounds differ from the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***) of the present invention in the following aspects of their composition:

SUMM 1. They are water-soluble agents, whereas DD-M. ***vaccae*** is insoluble in aqueous solutions.

SUMM . . . unit, either extracted from bacteria by various solvents, or digested from the cell wall by an enzyme. In contrast, DD-M. ***vaccae*** comprises processed mycobacterial cells.

SUMM . . . of the cell wall peptidoglycan structure, namely alanine, glutamic acid, diaminopimelic acid, N-acetyl glucosamine, and N-glycolylmuramic acid. In contrast, DD-M. ***vaccae*** contains 50% w/w protein, comprising a number of distinct protein species.

SUMM ***Asthma*** is a common disease, with a high prevalence in the developed world. ***Asthma*** is characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli, the primary physiological disturbance being reversible airflow limitation,

which may be spontaneous or drug-related, and the pathological hallmark being inflammation of the airways. Clinically, ***asthma*** can be subdivided into extrinsic and intrinsic variants.

SUMM Extrinsic ***asthma*** has an identifiable precipitant, and can be thought of as being atopic, occupational and drug-induced. Atopic ***asthma*** is associated with the enhancement of a Th2-type of immune response with the production of specific immunoglobulin E (IgE), positive. . . be divided further into seasonal and perennial forms according to the seasonal timing of symptoms. The airflow obstruction in extrinsic ***asthma*** is due to nonspecific bronchial hyperesponsiveness caused by inflammation of the airways. This inflammation is mediated by chemicals released by. . . and lymphocytes. The actions of these mediators result in vascular permeability, mucus secretion and bronchial smooth muscle constriction. In atopic ***asthma*** , the immune response producing airway inflammation is brought about by the Th2 class of T cells which secrete IL-4, IL-5 and IL-10. It has been shown that lymphocytes from the lungs of atopic ***asthmatics*** produce IL-4 and IL-5 when activated. Both IL-4 and IL-5 are cytokines of the Th2 class and are required for the production of IgE and involvement of eosimophils in ***asthma*** . Occupational ***asthma*** may be related to the development of IgE to a protein hapten, such as acid anhydrides in plastic workers and plicatic acid in some western red cedar-induced ***asthma*** , or to non-IgE related mechanisms, such as that seen in toluene diiusocyanate-induced ***asthma*** . Drug-induced ***asthma*** can be seen after the administration of aspirin or other non-steroidal anti-inflammatory drugs, most often in a certain subset of patients who may display other features such as nasal polyposis and sinusitis. Intrinsic or cryptogenic ***asthma*** is reported to develop after upper respiratory tract infections, but can arise de novo in middle-aged or older people, in whom it is more difficult to treat than extrinsic ***asthma*** .

SUMM ***Asthma*** is ideally prevented by the avoidance of triggering allergens but this is not always possible nor are triggering allergens always easily identified. The medical therapy of ***asthma*** is based on the use of corticosteroids and bronchodilator drugs to reduce inflammation and reverse airway obstruction. In chronic ***asthma*** , treatment with corticosteroids leads to unacceptable adverse side effects.

SUMM Another disorder with a similar immune abnormality to ***asthma*** is allergic rhinitis. Allergic rhinitis is a common disorder and is estimated to affect at least 10% of the population.. . .

SUMM . . . specific against the allergen. The inflammatory response occurs in the nasal mucosa rather than further down the airways as in ***asthma*** . Like ***asthma*** , local eosinophilia in the affected tissues is a major feature of allergic rhinitis. As a result of this inflammation, patients. . . and interfere with a person's ability to work. Current treatment involves the use of antihistamines, nasal decongestants and, as for ***asthma*** , sodium cromoglycate and corticosteroids.

SUMM . . . skin disease which usually occurs in families with an hereditary predisposition for various allergic disorders, such as allergic rhinitis and ***asthma*** . Atopic dermatitis occurs in approximately 10% of the general population. The main symptoms are dry skin, dermatitis (eczema) localised mainly. . .

SUMM . . . disorders, including disorders of the respiratory system (such as infection with mycobacteria such as M. tuberculosis or M. avium, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers), allergic disorders such as atopic dermatitis, diseases that benefit from the reduction of eosinophilia, and. . .

SUMM In a first aspect, compositions comprising ***delipidated*** and ***deglycolipidated*** mycobacterial cells are provided. In specific embodiments, the ***delipidated*** and ***deglycolipidated***

cells are prepared from M. ***vaccae*** , M. tuberculosis and/or M. smegmatis.

SUMM In a second aspect, the present invention provides compositions comprising a derivative of ***delipidated*** and ***deglycolipidated*** mycobacterial cells, the derivative of ***delipidated*** and ***deglycolipidated*** mycobacterial cells being selected from the group consisting of: ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated by alkaline hydrolysis; ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated by acid hydrolysis; and ***delipidated*** and ***deglycolipidated*** mycobacterial cells that have been treated with periodic acid. In preferred embodiments, such compositions comprise a derivative of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells, the derivative of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells being selected from the group consisting of: ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by alkaline hydrolysis; ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated by acid hydrolysis; and ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells that have been treated with periodic acid. The derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** contain glucosamine in an amount greater than 3.7% of total carbohydrate, preferably greater than 5% total carbohydrate and more preferably. . .

SUMM . . . a composition of the present invention. In certain embodiments, the disorder is selected from the group consisting of mycobacterial infections, ***asthma*** , sarcoidosis, allergic rhinitis and lung cancers. In one embodiment, the compositions are administered to the airways leading to or located. . .

SUMM . . . disorder is characterized by the presence of eosinophilia in the tissues of the respiratory system. Examples of such diseases include ***asthma*** and allergic rhinitis. In a related aspect, the present invention provides methods for the reduction of eosinophilia in a patient,. . .

DRWD FIG. 1 illustrates the induction of IL-12 by autoclaved M. ***vaccae*** , lyophilized M. ***vaccae*** , ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** , and M. ***vaccae*** glycolipids.

DRWD . . . stimulation of interferon-gamma production in spleen cells from Severe Combined ImmunoDeficient (SCID) mice by different concentrations of heat-killed (autoclaved) M. ***vaccae*** , ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** , and M. ***vaccae*** glycolipids.

DRWD FIG. 3 shows the suppression by DD-M. ***vaccae*** (Q.sub.1) and the DD-M. ***vaccae*** derivatives Q2 (DD-M. ***vaccae*** -KOH), Q3 (DD-M. ***vaccae*** -acid), Q4 (DD-M. ***vaccae*** -periodate), Q6 (DD-M. ***vaccae*** -KOH-periodate), P5 (DD-M. ***vaccae*** -KOH-acid) and P6 (DD-M. ***vaccae*** -KOH-periodate) of ovalbumin-induced airway eosinophilia in mice vaccinated intranasally with these compounds. Control mice received PBS.

DRWD FIG. 4 shows the stimulation of IL-10 production in THP-1 cells by derivatives of DD-M. ***vaccae*** .

DRWD FIG. 5 illustrates the effect of immunizing mice with heat-killed M. ***vaccae*** or ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***) prior to infection with tuberculosis.

DRWD FIG. 6 illustrates the re-suspension of DD-M. ***vaccae*** and DD-M.

vaccae -KOH.

DRWD FIG. 7 shows the stimulation of IL-12 production in macrophages by DD-M. ***vaccae*** (R1) and the DD-M. ***vaccae*** derivatives DD-M. ***vaccae*** -KOH (R2), (DD-M. ***vaccae*** -acid (R3), DD-M. ***vaccae*** -periodate (R4), DD-M. ***vaccae*** -KOH-acid (R5) and DD-M. ***vaccae*** -KOH-periodate (R6).

DRWD FIG. 8 illustrates the suppression of airway eosinophilia in a dose-dependent manner by a DD-M. ***vaccae*** -acid derivative.

DRWD FIG. 9 compares the effect of intranasal and intradermal immunization with DD-M. ***vaccae*** -acid on the suppression of lung eosinophils.

DRWD FIG. 10 illustrates the effect of immunization with DD-M. ***vaccae*** , DD-M. tuberculosis and DD-M. smegmatis on airway eosinophilia.

DRWD FIG. 11 illustrates TNF-.alpha. production by human PBMC and non-adherent cells stimulated with DD-M. ***vaccae*** .

DRWD FIGS. 12A and 12B illustrate IL-10 and IFN-.gamma. production, respectively, by human PBMC and non-adherent cells stimulated with DD-M. ***vaccae*** .

DETD . . . in which administration of IL-10 and/or stimulation of IL-10 production are beneficial. Examples of respiratory system disorders include mycobacterial infection, ***asthma*** , sarcoidosis, allergic rhinitis and lung cancer. Examples of disorders in which administration and/or increased production of IL-10 are believed to. . .

DETD . . . central to a reversal of disease state in many disorders, including disorders of the respiratory system such as tuberculosis, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers. IL-12 has been shown to up-regulate Th1 responses, while IL-10 has been shown to down-regulate. . . processes in the lung. (J. Clin. Invest. 1995, 95:2644-2651). Studies by Borish et al. have found that bronchoalveolar fluid from ***asthmatic*** patients contains reduced levels of IL-10 compared to that from normal donors (J. Allergy Clin. Immunol. 1996, 97:1288-96).

DETD In one aspect, methods are provided for the treatment of respiratory and/or lung disorders, comprising administering ***delipidated*** and ***deglycolipidated*** mycobacterial cells, preferably ***delipidated*** and ***deglycolipidated*** M. tuberculosis cells and/or ***delipidated*** and ***deglycolipidated*** M. smegmatis cells. In a related aspect, the present invention provides methods for the immunotherapy of respiratory and/or lung disorders, including tuberculosis, sarcoidosis, ***asthma*** , allergic rhinitis and lung cancers, in a patient by administration of a composition that comprises at least one derivative of ***delipidated*** and ***deglycolipidated*** mycobacterial cells. In certain specific embodiments, such methods comprise administering at least one derivative of DD-M. ***vaccae*** . As detailed below, the inventors have demonstrated that administration of such compositions is effective in the treatment of ***asthma*** in a mouse model. These compositions are believed to be effective in the treatment of diseases such as ***asthma*** due to their ability to suppress ***asthma*** -inducing Th2 immune responses. In one embodiment, the compositions are delivered directly to the mucosal surfaces of airways leading to and/or. . .

DETD As used herein the term "inactivated M. ***vaccae*** " refers to M. ***vaccae*** cells that have either been killed by means of heat, as detailed below in Example 1, or subjected to radiation, such as .sup.60Cobalt at a dose of 2.5 megarads. As used herein, the term "modified M. ***vaccae*** " includes ***delipidated*** M. ***vaccae*** cells, ***deglycolipidated*** M. ***vaccae*** cells, M. ***vaccae*** cells that have been both ***delipidated*** and ***deglycolipidated*** (DD-M. ***vaccae***), and derivatives of ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** cells. DD-M. ***vaccae*** may be prepared as described below in Example 1, with the preparation of derivatives of DD-M. ***vaccae*** being detailed below in Example 2. The

preparation of ***delipidated*** and ***deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis) is described in Example 10 below. Derivatives of DD-M. tuberculosis and. . . acid-treated, alkali-treated and/or periodate-treated derivatives, may be prepared using the procedures disclosed herein for the preparation of derivatives of DD-M. ***vaccae*** .

DETD The derivatives of DD-M. ***vaccae*** preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of DD-M. ***vaccae*** preferably contain glucosamine in an amount greater than 3.7% of total carbohydrate, more preferably greater than 5% total carbohydrate, and most preferably greater than 7.5% total carbohydrate. Derivatives prepared by treatment of DD-M. ***vaccae*** with alkali, such as DD-M. ***vaccae*** -KOH have a reduced number of ester bonds linking mycolic acids to the arabinogalactan of the cell wall compared to DD-M. ***vaccae*** . Derivatives prepared by treatment with acid, such as DD-M. ***vaccae*** -acid, have a reduced number of phosphodiester bonds attaching arabinogalactan sidechains to the peptidoglycan of the cell wall. Derivatives prepared by treatment of DD-M. ***vaccae*** with periodate, such as DD-M. ***vaccae*** -periodate, have a reduced number of cis-diol-containing sugar residues compared to DD-M. ***vaccae*** .

DETD . . . in an aerosol form or by means of a nebulizer device similar to those currently employed in the treatment of ***asthma*** .

DETD . . . and a non-specific stimulator of immune responses, such as lipid A, Bordetella pertussis, M. tuberculosis, or, as discussed below, M. ***vaccae*** . Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, Mich.), and. . .

DETD The preferred frequency of administration and effective dosage will vary from individual to individual. For both DD-M. ***vaccae*** and derivatives of DD-M. ***vaccae*** , the amount present in a dose preferably ranges from about 10 .mu.g to about 1000 .mu.g, more preferably from about. . .

DETD Preparation of ***Delipidated*** and ***Deglycolipidated*** M. ***vaccae*** Cells (DD-M. ***vaccae***)

DETD This example illustrates the processing of different constituents of M. ***vaccae*** and their immune modulating properties.

DETD Heat-killed M. ***vaccae*** and M. ***vaccae*** culture filtrate

DETD M. ***vaccae*** (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose 1 g/l) at. . . removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10.sup.10 M. ***vaccae*** organisms per ml. The cell suspension was then autoclaved for 15 min at 120.degree. C. The culture filtrate was passaged. . .

DETD Preparation of ***Delipidated*** and ***Deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***) and Compositional Analysis

DETD To prepare ***delipidated*** M. ***vaccae*** , the autoclaved M. ***vaccae*** was pelleted by centrifugation, the pellet washed with water and collected again by centrifugation, and freeze-dried. An aliquot of this freeze-dried M. ***vaccae*** was set aside and referred to as lyophilised M. ***vaccae*** . When used in experiments it was resuspended in PBS to the desired concentration. Freeze-dried M. ***vaccae*** was treated with chloroform/methanol (2:1) for 60 mins at room temperature to extract lipids, and the extraction was repeated once. The ***delipidated*** residue from the chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of M. ***vaccae*** glycolipids (see below). The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of ***delipidated*** and ***deglycolipidated*** M. ***vaccae***

prepared was equivalent to 11.1% of the starting wet weight of M. ***vaccae*** used. For bioassay, the ***delipidated*** and ***deglycolipidated*** M. ***vaccae*** (DD-M. ***vaccae***), was resuspended in phosphate-buffered saline by sonication, and sterilised by autoclaving.

DETD The compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae*** are presented in Table 1. Major changes are seen in the fatty acid composition and amino acid composition of DD-M. ***vaccae*** as compared to the insoluble fraction of heat-killed M. ***vaccae*** . The data presented in Table 1 show that the insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-M. ***vaccae*** contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

DETD

TABLE 1

Compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae***

MONOSACCHARIDE COMPOSITION

sugar alditol	M. ***vaccae***	DD-M. ***vaccae***
Inositol	3.2%	1.7%
Ribitol*	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%

DETD

TABLE 1

Compositional analyses of heat-killed M. ***vaccae*** and DD-M. ***vaccae***

MONOSACCHARIDE COMPOSITION

sugar alditol	M. ***vaccae***	DD-M. ***vaccae***
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Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%

DETD The insoluble fraction of heat-killed M. ***vaccae*** contains 10% w/w of lipid, and DD-M. ***vaccae*** contains 1.3% w/w of lipid.

DETD

AMINO ACID COMPOSITION

nmoles/mg	M. ***vaccae***	DD-M. ***vaccae***
ASP	231	361
THR	170	266
SER	131	199
GLU	319	505
PRO	216	262
GLY	263	404
ALA.	.	.

DETD The total amino acid content of the insoluble fraction of heat-killed M. ***vaccae*** is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-M. ***vaccae*** is 4250 nmoles/mg, or approximately 51% w/w.

DETD M. ***vaccae*** glycolipids

DETD . . . evaporation, redissolved in water, and freeze-dried. The amount of glycolipid recovered was 1.2% of the starting wet weight of M.

vaccae used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

DETD Whole heat-killed M. ***vaccae*** and DD-M. ***vaccae*** were shown to have different cytokine stimulation properties. The stimulation of a Th1 immune response is enhanced by the production of interleukin-12 (IL-12) from macrophages. The ability of different M. ***vaccae*** preparations to stimulate IL-12 production was demonstrated as follows.

DETD . . . cell culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of whole heat-killed (autoclaved) M. ***vaccae*** , lyophilized M. ***vaccae*** , DD-M. ***vaccae*** (referred to as ***delipidated*** - ***deglycolipidated*** M. ***vaccae*** in FIG. 1) and M. ***vaccae*** glycolipids were added. After a further three days at 37.degree. C., the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in FIG. 1, the M. ***vaccae*** preparations stimulated the production of IL-12 from macrophages.

DETD By contrast, these same M. ***vaccae*** preparations were examined for the ability to stimulate interferon-gamma (IFN-.gamma.) production from Natural Killer (NK) cells. Spleen cells were prepared. . . contain 75-80% NK cells. The spleen cells were incubated at 37.degree. C. in culture with different concentrations of heat-killed M. ***vaccae*** , DD-M. ***vaccae*** , or M. ***vaccae*** glycolipids. The data shown in FIG. 2 demonstrates that, while heat-killed M. ***vaccae*** and M. ***vaccae*** glycolipids stimulate production of interferon-gamma, DD-M. ***vaccae*** stimulated relatively less interferon-gamma. The combined data from FIGS. 1 and 2 indicate that, compared with whole heat-killed M. ***vaccae*** , DD-M. ***vaccae*** is a better stimulator of IL-12 than of interferon-gamma.

DETD Preparation and Characterisation of Additional Derivates M. ***vaccae***

DETD Alkaline Hydrolysis of DD-M. ***vaccae***

DETD One gram of DD-M. ***vaccae*** , prepared as described in Example 1, was suspended in 20 ml of a 0.5% solution of potassium hydroxide (KOH) in. . . ethanol and once with diethyl ether. The product was air-dried overnight. The yield was 1.01 g (101%) of KOH-treated DD-M. ***vaccae*** , subsequently referred to as DD-M. ***vaccae*** -KOH. This derivative was found to be more soluble than the other derivatives of DD-M. ***vaccae*** disclosed herein.

DETD Acid Hydrolysis of DD-M. ***vaccae***

DETD DD-M. ***vaccae*** or DD-M. ***vaccae*** -KOH (100 mg) was washed twice in 1 ml of 50 mM H.sub.2SO.sub.4 followed by resuspension and centrifugation. Other acids are. . . acid was removed by washing the residue five times with water. The freeze-dried solid residue yielded 58.2 mg acid-treated DD-M. ***vaccae*** (DD-M. ***vaccae*** -acid) or 36.7 mg acid-treated DD-M. ***vaccae*** -KOH (DD-M. ***vaccae*** -KOH-acid).

DETD Periodic Acid Cleavage of DD-M. ***vaccae***

DETD This procedure is intended to cleave cis-diol-containing sugar residues in DD-M. ***vaccae*** , such as the rhamnose residue near the attachment site of the arabinogalactan chains to the peptidoglycan backbone.

DETD DD-M. ***vaccae*** or DD-M. ***vaccae*** -KOH (100 mg) was suspended in 1 ml of a solution of 1% periodic acid in 3% acetic acid, incubated for. . . centrifugation, the solid residue was washed four times with water and freeze-dried to give a yield of 62.8 mg DD-M. ***vaccae*** -periodate or 61.0 mg DD-M. ***vaccae*** -KOH-periodate.

DETD Resuspension of DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH

DETD DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH (11 mg each) were suspended in phosphate-buffered saline (5.5 ml). Samples were sonicated with a Virtis probe sonicator for various. . . of large particles. The absorbance of the remaining suspension at 600 nm was measured. As

shown in FIG. 6, DD-M. ***vaccae*** -KOH (referred to in FIG. 6 as DDMV-KOH) was fully resuspended after one minute's sonication and further sonication produced no further increase in the absorbance. After five minutes sonication, the resuspension of DD-M. ***vaccae*** (referred to in FIG. 6 as DDMV) was still incomplete as estimated from the absorbance of the suspension. These results indicate that DD-M. ***vaccae*** -KOH is considerably more soluble than DD-M. ***vaccae*** .

DETD Effect of Immunisation with DD-M. ***vaccae*** and Derivates of DD-M. ***vaccae*** on ***Asthma*** in Mice

DETD The ability of DD-M. ***vaccae*** and derivatives of DD-M. ***vaccae*** to inhibit the development of allergic immune responses was examined in a mouse model of the ***asthma*** -like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate. . .

DETD DD-M. ***vaccae*** derivatives were prepared as described above. Groups of 10 mice were administered 200 .mu.g of PBS, DD-M. ***vaccae*** or one of the DD-M. ***vaccae*** derivatives (Q1: DD-M. ***vaccae*** ; Q2: DD-M. ***vaccae*** -KOH; Q3: DD-M. ***vaccae*** -acid; Q4: M. ***vaccae*** -periodate; Q6 and P6: DD-M. ***vaccae*** -KOH-periodate; P5: DD-M. ***vaccae*** -KOH-acid) intranasally one week before intranasal challenge with ovalbumin. As shown in FIG. 3, statistically significant reductions were observed in the. . . days after challenge with ovalbumin, compared to control mice. Furthermore, the data shows that suppression of airway eosinophilia with DD-M. ***vaccae*** -acid and DD-M. ***vaccae*** -KOH-periodate (Q3, Q6 and P6) was greater than that obtained with DD-M. ***vaccae*** (Q1). Control mice were given intranasal PBS. The data in FIG. 3 shows the mean and SEM per group of. . .

DETD Eosinophils are blood cells that are prominent in the airways in allergic ***asthma*** . The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic ***asthma*** . The data shown in FIG. 3 indicate that treatment with DD-M. ***vaccae*** or derivatives of DD-M. ***vaccae*** reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-M. ***vaccae*** or derivatives of DD-M. ***vaccae*** may therefore reduce the severity of ***asthma*** and diseases that involve similar immune abnormalities, such as allergic rhinitis.

DETD In addition, serum samples were collected from mice immunized with either heat-killed M. ***vaccae*** or DD-M. ***vaccae*** and the level of antibodies to ovalbumin was measured by standard enzyme-linked immunoassay (EIA). As shown in Table 2 below,. . . BCG had higher levels of ovalbumin specific IgG1 than sera from PBS controls. In contrast, mice immunized with heat-killed M. ***vaccae*** or DD-M. ***vaccae*** had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of DD-M. ***vaccae*** on the ***asthma*** -inducing Th2 immune responses.

DETD

TABLE 2

Low Antigen-Specific IgG1 Serum Levels in Mice Immunized with Heat-killed M. ***vaccae*** or DD-M. ***vaccae***

Treatment Group			Serum IgG1 Mean	SEM
M.	***vaccae***	i.n.	185.00	8.3
M.	***vaccae***	s.c.	113.64	8.0
DD-M.	***vaccae***	i.n.	96.00	8.1
DD-M.	***vaccae***	s.c.	110.00	4.1
BCG, Pasteur			337.00	27.2

BCG, Connaught 248.00 46.1
PBS 177.14 11.4

DETD Effect of DD-M. ***vaccae*** Derivates on IL-10 Production in THP-1 Cells

DETD . . . The levels of IL-10 produced by a human monocytic cell line (THP-1) cultured in the presence of derivatives of DD-M. ***vaccae*** were assessed as follows.

DETD . . . cell concentration and viability determined by staining with Trypan blue (Sigma, St Louis Mich.) and analysis under a hemocytometer. DD-M. ***vaccae*** derivatives (prepared as described above) in 50 .mu.l PBS and control stimulants were added in triplicate to wells of a. . . San Diego Calif.) according to the manufacturer's protocol. As shown in FIG. 4, the acid and periodate derivatives of DD-M. ***vaccae*** were found to stimulate significant levels of IL-10 production. The PBS control, DD-M. ***vaccae*** -KOH, DD-M. ***vaccae*** -KOH-periodate, and DD-M. ***vaccae*** -KOH-acid derivatives did not stimulate THP-1 cells to produce IL-10.

DETD Effect of Immunizing Mice with M. ***vaccae*** , and DD-M. ***vaccae*** on Tuberculosis

DETD This example illustrates the effect of immunization with heat-killed M. ***vaccae*** or DD-M. ***vaccae*** prior to challenge with M. tuberculosis.

DETD b) Heat-killed M. ***vaccae*** (500 .mu.g); and

DETD c) DD-M. ***vaccae*** (50 .mu.g).

DETD . . . CFU below the baseline for control mice (or log protection). As shown in FIG. 5, mice immunized with heat-killed M. ***vaccae*** or DD-M. ***vaccae*** showed respectively a mean reduction of >1 or 0.5 logs CFU. The data demonstrates the effectiveness of immunization with M. ***vaccae*** or DD-M. ***vaccae*** and indicates that DD-M. ***vaccae*** may be developed as a vaccine against tuberculosis.

DETD Compositional Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** Derivates

DETD Carbohydrate compositional analysis of DD-M. ***vaccae*** and DD-M ***vaccae*** derivatives

DETD The carbohydrate composition of DD-M. ***vaccae*** and DD-M. ***vaccae*** derivatives was determined using standard techniques. The results are shown in Table 3, wherein DDMV represents DD-M. ***vaccae*** ; DDMV-KOH represents DD-M. ***vaccae*** -KOH; DDMV-A represents DD-M. ***vaccae*** -acid; DDMV-I represents DD-M. ***vaccae*** -periodate; DDMV-KOH-A represents DD-M. ***vaccae*** -KOH-acid; and DDMV-KOH-I represents DD-M. ***vaccae*** -KOH-periodate.

DETD
TABLE 3

Carbohydrate Compositional Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** Derivatives

Carbohydrate	DDMV	DDMV- KOH	DDMV-A	DDMV-I	KOH-A	KOH-I
Galactosamine	26.6*	29.2	14.9	37.7	0.3	3.9
Glucosamine	3.7	3.6	8.7	35.6.	.	.

DETD The results demonstrate that each of the DD-M. ***vaccae*** derivatives had a different carbohydrate content, as expected from the different effects of the acid, periodate or alkali treatment of the cells. In addition, DD-M. ***vaccae*** had a marked different carbohydrate composition when compared with the DD-M. ***vaccae*** derivatives. As expected, the amount of galactose in the DD-M. ***vaccae*** -acid and DD-M. ***vaccae*** -periodate derivatives was lower than in DD-M. ***vaccae*** and DD-M. ***vaccae*** -KOH. These values reflect the action of the acid and periodate in the preparation of the derivatives, cleaving the arabinogalactan sidechains. . .

DETD Nucleic Acid Analysis of DD-M. ***vaccae*** and DD-M. ***vaccae*** derivatives

DETD Analysis by gel electrophoresis of the nucleic acid content of DD-M. ***vaccae*** and the DD-M. ***vaccae*** derivatives after treatment with Proteinase K showed that DD-M. ***vaccae*** , DD-M. ***vaccae*** -periodate and DD-M. ***vaccae*** -KOH contained small amounts of DNA while no. detectable nucleic acid was observed for DD-M. ***vaccae*** -acid.

DETD Effect of DD-M. ***vaccae*** Derivates on IL-12 Production by Macrophages

DETD . . . of a Th1 immune response is enhanced by the production of interleukin-12 (IL-12) from macrophages. The ability of different M. ***vaccae*** preparations to stimulate IL-12 production was demonstrated as follows.

DETD . . . M 2-mercaptoethanol and 5% fetal bovine serum (FBS), and adjusted to a concentration of 2.times.10.sup.6 cells/ml. Various concentrations of DD-M. ***vaccae*** (referred to in FIG. 7 as R1) and DD-M. ***vaccae*** -KOH (referred to in FIG. 7 as R2), DD-M. ***vaccae*** -acid (referred to in FIG. 7 as R3), DD-M. ***vaccae*** -periodate (referred to in FIG. 7 as R4), DD-M. ***vaccae*** -KOH-acid (referred to in FIG. 7 as R5), and DD-M. ***vaccae*** -KOH-periodate (referred to in FIG. 7 as P6) were added. To each well, 0.1 ml of IFN-.gamma.-treated macrophages were added at. . . assayed for the presence of IL-12 produced by macrophages. The level of IL-12 production by macrophages stimulated with the DD-M. ***vaccae*** derivatives are shown in FIG. 7. The data indicates that the DD-M. ***vaccae*** derivatives stimulated IL-12 production by macrophages at approximately the same level as DD-M. ***vaccae*** , with the exception of DD-M. ***vaccae*** -KOH-acid, which induced less IL-12 production.

DETD Effect of Immunizing Mice with Different Dosages of DD-M. ***vaccae*** Derivates

DETD This example illustrates the effect of immunization with different dosages of DD-M. ***vaccae*** derivatives on the development of an allergic immune response in the lungs. This was demonstrated in a mouse model of the ***asthma*** -like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate. . .

DETD . . . and 7. On days 14 and 21, mice were anesthetized and vaccinated intranasally or intradermally with 200 .mu.g of DD-M. ***vaccae*** -acid or PBS. On days 28 and 32, mice were anesthetized and challenged intranasally with 100 .mu.g OVA. Mice were sacrificed. . .

DETD As can be seen in FIG. 8, DD-M. ***vaccae*** -acid caused a statistically significant, dosage-dependent suppression of airway eosinophilia (% eosinophils), with increasing levels of suppression being observed with increasing dosages of DD-M. ***vaccae*** -acid.

DETD Effects of the Route of Immunization of Mice with Derivates of DD-M. ***vaccae***

DETD This example illustrates the effect of different routes of immunization with DD-M. ***vaccae*** derivatives on the suppression of eosinophilia in the lung in a mouse model of the ***asthma*** -like allergen specific lung disease.

DETD . . . and 7. On days 14 and 21, mice were anesthetized and vaccinated intranasally or intradermally with 200 .mu.g of DD-M. ***vaccae*** -acid or PBS. On days 28 and 32, mice were anesthetized and challenged intranasally with 100 .mu.g OVA. Mice were sacrificed. . .

DETD . . . the percentage of eosinophils in BAL cells collected six days after challenge with ovalbumin from mice immunized intranasally with DD-M. ***vaccae*** -acid, compared to control mice. Furthermore, the data shows that suppression of airway eosinophilia with DD-M. ***vaccae*** -acid administered intranasally was greater than that when mice were immunized intradermally. Control mice were given intranasal PBS. The data in. . .

DETD Preparation and Compositional Analisys of ***Delipidated*** and ***Deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis)

DETD Cultures of Mycobacterium smegmatis (M. smegmatis, ATCC Number 27199) were grown as described in Example 1 for M. ***vaccae*** in Medium 90 with 1% added glucose. After incubation at 37.degree. C. for 5 days, the cells were harvested by. . .

DETD Preparation of ***Delipidated*** and ***Deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and ***Delipidated*** and ***Deglycolipidated*** M. smegmatis (DD-M. smegmatis) and Compositional Analysis

DETD To prepare ***delipidated*** and ***deglycolipidated*** M. tuberculosis (DD-M. tuberculosis) and M. smegmatis (DD-M. smegmatis), autoclaved M. tuberculosis and M. smegmatis were pelleted by centrifugation, the. . .

DETD ***Delipidated*** and ***deglycolipidated*** M. tuberculosis and M. smegmatis were prepared as described in Example 1 for the preparation of DD-M. ***vaccae*** . For bioassay, the freeze-dried DD-M. tuberculosis and DD-M. smegmatis were resuspended in phosphate-buffered saline (PBS) by sonication, and sterilized by. . .

DETD . . . in some components of the monosaccharide composition of DD-M. tuberculosis and DD-M. smegmatis compared with the monosaccharide composition of DD-M. ***vaccae*** . The data presented in Table 4 show that DD-M. tuberculosis and DD-M. smegmatis contain 1.3% and 0.0 mol % glucose, respectively, compared with 28.1 mol % for DD-M. ***vaccae*** .

DETD . . . contains 6007.7 nmoles/mg amino acids, which is approximately 72.1% w/w protein. When compared with the amino acid analysis of DD-M. ***vaccae*** given in Table 1, DD-M. tuberculosis and DD-M. smegmatis contain more total % protein than DD-M. ***vaccae*** (55.1%).

DETD Effect of Immunization with DD-M. tuberculosis and DD-M. smegmatis on ***Asthma*** in Mice

DETD . . . tuberculosis and DD-M. smegmatis to inhibit the development of allergic immune responses was examined in a mouse model of the ***asthma*** -like allergen specific lung disease, as described above in Example 8. The results illustrates the effect of immunization with DD-M. tuberculosis. . .

DETD . . . and 7. On days 14 and 21, mice were anesthetized and vaccinated intranasally or intradermally with 200 .mu.g of DD-M. ***vaccae*** , DD-M. tuberculosis, DD-M. smegmatis or PBS. On days 28 and 32, mice were anesthetized and challenged intranasally with 100 .mu.g. . .

DETD . . . with DD-M. tuberculosis and DD-M. smegmatis reduces the accumulation of lung eosinophils similar to the reduction following immunization with DD-M. ***vaccae*** , and that DD-M. tuberculosis and DD-M. smegmatis may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-M. tuberculosis and DD-M smegmatis may therefore reduce the severity of ***asthma*** and diseases that involve similar immune abnormalities, such as allergic rhinitis.

DETD Effect of DD-M. ***vaccae*** on Production of IL-10, TNF-Alpha and INF-Gamma in Human Peripheral Blood Mononuclear Cells

DETD This example describes studies on the ability of DD-M. ***vaccae*** to stimulate cytokine production in human peripheral blood mononuclear cells (PBMC).

DETD . . . blood was separated into PBMC and non-adherent cells, and the cytokine production of each fraction determined after stimulation with DD-M. ***vaccae*** as follows. Blood was diluted with an equal volume of saline and 15-20 ml was layered onto 10 ml Ficoll. . .

DETD . . . were removed, counted and resuspended at a concentration of 2.times.10.sup.6 per ml in supplemented RPMI medium. Serial dilutions of DD-M. ***vaccae*** were prepared starting at 200 .mu.g/ml and added to 100 .mu.l medium (supplemented RPMI) in a 96-well plate. PBMC and. . . was removed from each well to determine the amount of cytokine

produced by the different cells after stimulation with DD-M. ***vaccae*** .

DETD DD-M. ***vaccae*** stimulated PBMC to secrete TNF-.alpha. and IL-10 (FIGS. 11 and 12A, respectively), but stimulated the non-adherent cells to produce IFN-.gamma. (FIG. 12B). These data suggest that IFN-.gamma. production in DD-M. ***vaccae*** -stimulated PBMC is repressed by the simultaneous secretion of IL-10.

DETD Activation of T Cells by Heart-killed M. ***vaccae*** and DD-M. ***vaccae***

DETD The ability of heat-killed M. ***vaccae*** and DD-M. ***vaccae*** to activate human T cells and Natural Killer (NK) cells was examined as follows.

DETD . . . mononuclear cells (PBMC) at a concentration of 5.times.10.sup.6 cells per ml were cultured with 20 ug/ml of either heat-killed M. ***vaccae*** or DD-M. ***vaccae*** for 24 hours. Control cells were cultured with media alone. Cultured cells were then stained with monoclonal antibodies against CD56. . .

DETD

TABLE 6

Activation of Human T Cells and NK Cells by Heat-Killed M. ***vaccae*** and DD-M. ***vaccae***

	.alpha..beta.T cells	.gamma..delta.T cells	NK cells
Control	3.8	6.2	4.8
Heat-killed M. ***vaccae***	8.3	10.2	40.3
DD-M. ***vaccae***	10.1	17.5	49.9

DETD These results indicate that heat-killed M. ***vaccae*** and DD-M. ***vaccae*** activate both .alpha..beta. and .gamma..delta.T cells, as well as NK cells.

DETD Recent studies by Holt and Sly (Nature Medicine, 1999, 5:1127-1128) indicate that, in ***asthma*** , .gamma..delta.T cells are important in maintaining normal airway responsiveness and downregulate airway responsiveness to allergen challenge, possibly by controlling the "repair" response of the airway epithelium to .gamma..delta.T cells cell-mediated damage. Since M. ***vaccae*** and DD-M. ***vaccae*** are able to activate .gamma..delta.T cells, they are likely to effective in restoring a normal epithelium in diseased areas of. . .

CLM What is claimed is:

1. A composition comprising ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** cells that have been treated by acid hydrolysis.

2. The composition of claim 1, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by acid hydrolysis contain galactose in an amount less than 9.7% of total carbohydrate.

3. The composition of claim 1, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by acid hydrolysis contain glucosamine in an amount greater than 3.7% of total carbohydrate.

4. The composition of claim 1, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by acid hydrolysis contain galactosamine in an amount less than 26.6% of total carbohydrate.

5. The composition of claim 1, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by acid hydrolysis contain glucose in an amount greater than 56.9% of total carbohydrate.

6. The composition of claim 1, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by acid hydrolysis contain mannose in an amount greater than 3.2% of total carbohydrate.

8. A composition comprising ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** cells that have been treated with periodic acid.

9. The composition of claim 8, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated with periodic acid contain galactose in an amount less than 9.7% of total carbohydrate.

10. The composition of claim 8, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated with periodic acid contain glucosamine in an amount greater than 3.7% of total carbohydrate.

12. A composition comprising ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis.

13. The composition of claim 12, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis contain galactose in an amount less than 9.7%. . .

14. The composition of claim 12, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and by acid hydrolysis contain glucosamine in an amount greater than 3.7%. . .

16. A composition comprising ***delipidated*** and ***deglycolipidated*** Mycobacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid.

17. The composition of claim 16, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid contain galactose in an amount less than. . .

18. The composition of claim 16, wherein the ***delipidated*** and ***deglycolipidated*** Microbacterium ***vaccae*** cells that have been treated by alkaline hydrolysis and treated with periodic acid contain glucosamine in an amount greater than. . .